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Rooting Characteristics and Antioxidant Pigment Responses of Three Hybrid Bermudagrass [*Cynodon dactylon* (L.) Pers. × *Cynodon transvaalensis* Burtt- Davy] Cultivars Exposed to Drought

Cory Alan Yurisic
University of Tennessee - Knoxville, cyurisic@vols.utk.edu

To the Graduate Council:

I am submitting herewith a thesis written by Cory Alan Yurisic entitled "Rooting Characteristics and Antioxidant Pigment Responses of Three Hybrid Bermudagrass [*Cynodon dactylon* (L.) Pers. × *Cynodon transvaalensis* Burtt- Davy] Cultivars Exposed to Drought." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

John C. Sorochan, Major Professor

We have read this thesis and recommend its acceptance:

Jim T. Brosnan, Dean A. Kopsell, John C. Stier

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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DEDICATION

To my parents, Karen and Mark for their support in my decision to return to school to pursue my passion.

To my beloved best friend, Whitney. Thank you so much for always being there to encourage me during the times when I felt overwhelmed.

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ABSTRACT

With recent changes in global climate, water use and its conservation have become important topics of discussion. Turfgrasses for lawns, parks, and other recreational areas are often perceived negatively due to the need for irrigation. The identification of turfgrasses possessing an improved ability to withstand water deficit is an important goal in science to curb irrigation requirements in the landscape. Newly released cultivars of hybrid bermudagrass (*C. dactylon* L. x. *C. transvaalensis* Burtt-Davy) have demonstrated improved tolerance to stresses such as cold, traffic, and water deficit; however, drought resistance mechanisms of these new cultivars have been largely uninvestigated. Drought resistant plant species are often characterized by deep and extensive root systems and improved antioxidant defense mechanisms that can reduce the damaging effects of reactive oxygen species. Several studies in a variety of different species of turfgrass have focused on root architecture, while others have examined changes in enzymatic antioxidants. However, investigations into the roles of antioxidant pigments in turfgrasses exposed to stress are quite limited. This research examined drought resistance aspects of three commonly known hybrid bermudagrass cultivars. Experiments at two locations utilizing lysimeter-grown plants were designed to evaluate differences in rooting characteristics and changes in antioxidant pigment composition among cultivars exposed to drought stress. Hybrid bermudagrass plants were grown in 45-cm tall lysimeters and subjected to 28 days of drought stress under natural field conditions by withholding all water during the summer of 2015. Lysimeters were arranged in a completely randomized experimental design with three replications at two locations. Pigments were extracted from leaf tissue using high performance liquid chromatography at four day intervals during the experiments. At the end of the study, plant roots were washed and analyzed for root length, mass, and architecture for three

separate soil depths. Cultivars exhibited significant differences for antioxidant pigment composition and rooting characteristics. Cultivars evaluated in this study included ‘TifTuf’, ‘Latitude 36’, and ‘Tifway’. The TifTuf cultivar demonstrated superior drought resistance which was characterized by increased root weight, surface area, and volume at deeper soil depths, and greater antioxidant pigment concentrations and xanthophyll cycle activity at the later stages of drought.

PREFACE

"I have written of dawn, of the moon, and the trees;

Of people, and flowers, and the songs of the bees;

But over those things my mind would pass,

And come to rest among the grass."

-Mable Duggan

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CHAPTER I - LITERATURE REVIEW

Introduction

Water Issues and Use in the U.S.

Water is one of the most essential molecules used by plants and animals to support growth and metabolism, and is a vital key to sustaining life on Earth. Water is important to plants due to its crucial role in nearly all physiological processes, and comprises approximately 70-95% of the biomass of leaf and root tissues (Lambers et al., 2008). It is an excellent solvent; thereby making it an ideal medium for the movement and transport of molecules between cells in addition to dissolved soil minerals from the roots to the leaves. Water makes up the environment in which biochemical reactions occur and also plays a direct role in many of these reactions (Taiz and Zeiger, 2002). Without water, plants would not be able to carry out the reactions involved in photosynthesis that provide the oxygen that life on Earth requires to survive.

In the United States, there are an estimated 50 million acres of maintained turfgrass including home lawns, golf courses, sports fields, parks, playgrounds, cemeteries, and highway rights of way. The estimated annual economic value of these areas is estimated to be $40 billion (Fender, 2006). According to the United States Geological Survey (USGS), it is estimated that on Earth there are 332.5 million cubic miles of water, of which only 2.5% is freshwater. And of that fraction, only 32% is readily available to humans either as groundwater, or embodied in lakes and rivers (USGS, 2014). On the positive side, a multi-year survey published by the USGS found that freshwater withdrawals for all purposes in the United States has decreased by approximately 5% since the 1980's to nearly 410 billion gallons of water per day. Approximately 1% of that amount was used for domestic purposes including residential irrigation, and another 37% was utilized for irrigation on golf courses, parks, nurseries, and cropland (Kenny et al.,

2009). However, even though water use has decreased over time, as the population of the United States continues to increase, it is difficult to predict whether that trend will continue into the future.

Currently, the areas in the United States under the greatest water stress are the Midwest, and California, which coincides with those areas having the highest demand for water for use in agriculture and municipal purposes (Shi et al., 2013). Although precise numbers for how much irrigation water is actually being used for each individual agricultural purpose are very difficult to assess, the turfgrass industry is often scrutinized for wasteful use of water. In some areas like Nevada, the Southern Nevada Water Authority has initiated a program that will pay homeowners $1.50 for each square foot of turfgrass removed and replaced by xeriscape landscaping (SNWA, 2014). In other places such as California, water for homeowner lawn irrigation is strictly regulated, with fines levied if certain guidelines are violated. There are no nationwide water use regulations as water issues are considered to be local issues (Richardson, 2006); however, this could change in the future as water quality and quantity continues to become a bigger issue.

It is important to recognize that whether turfgrass systems are an actual cause of strain on the country's water supply or not, a well-kept stand of turf can provide a wide variety of functional, recreational, and aesthetic benefits that warrant their use in the landscape. According to Beard and Green (1994), the ambient temperature of a green bermudagrass (*Cynodon spp.)* turfgrass area is up to 21 °C less than a brown dormant turf due to transpirational cooling effects. This can result in lower home cooling energy costs in the summertime. Another important benefit of turfgrass is the prevention of soil erosion due to its high shoot density and root mass that stabilizes the soil, and resists lateral surface water flow (Beard and Green, 1994). Furthermore, properly managed turfgrasses offer a safe and reliable playing surface for sports

and other recreational activities. It is for reasons such as these that turfgrass areas provide important functions in urban landscapes.

Another important consideration is the fact that water use for irrigation purposes is ultimately in the hands of the turf manager or homeowner to apply irrigation in a responsible manner to avoid over-watering. One way this can achieved is by more efficient irrigation system design, and also by making more precise site-specific water applications (Carrow et al., 2002). Another way is by understanding the water needs of the turfgrass species being managed, and selecting the correct species for the location. According to Beard and Kim (1989), the goal of selecting the proper turfgrass should be to select species that require the least possible supplemental irrigation for the climate. These grasses are typically characterized by high shoot density, high leaf number, horizontal leaf orientation, slow vertical leaf extension rate, and narrow leaves (Beard and Kim, 1989). In 1989, researchers at Texas A&M University evaluated the water use rates of 19 commonly used turfgrass species throughout the United States (Beard and Kim, 1989). Results indicated that there are considerable differences among water use rates among the most common cool and warm-season turfgrasses, which should be an important consideration when making a turfgrass selection. Combining responsible irrigation practices with proper turfgrass selection are practical measures that can be taken to decrease water consumption.

It should be noted that reduced water use in plants does not necessarily mean that the plant will be more drought tolerant, but rather it can survive on a given amount of water longer than a plant that uses more water. Therefore, in dry climates when supplemental irrigation is limited or completely unavailable, a turf manager must make more effort to select drought tolerant and drought resistant species and cultivars to use for a landscape (Shearman, 1985). As

water use and availability continues to become a major concern, the need for more drought resistant species and cultivars will increase.

Bermudagrass

One such species of turfgrass that possesses an innate ability to withstand water deficit is bermudagrass (*Cynodon spp.*). According to Harlan (1970), there exists eight different classified *Cynodon* species. Of these, only two are considered turfgrasses (common bermudagrass, *C. dactylon* (L.) Pers. var. *dactylon,* and African bermudagrass, *C. transvaalensis* Burt-Davy (Casler and Duncan, 2003)). *C. dactylon* var. *dactylon* is believed to have been introduced into the United States in the mid 1700's where it became readily dispersed and naturalized, whereas *C. transvaalensis* was most likely introduced much later by human travelers from South Africa (Beard, 2012). Interspecific hybrid crosses between the two species have increased the potential of bermudagrass utilization in the United States.

Although *C. transvaalensis* has been used on some sporting surfaces, its high water and fertility requirements, and its intolerance to low mowing heights has limited its use as a turfgrass (Wu, Taliaferro, et al., 2005). Interspecific hybridization between *C. dactylon* and *C. transvaalensis* has resulted in the introduction of many new hybrid cultivars, whose diverse traits can vary enormously (Taliaferro, 1995). Combining the aggressive growth habit of tetraploid *C. dactylon* with the fine textured diploid *C. transvaalensis* yields sterile triploid hybrids with intermediate growth habit and leaf texture ideal for the golf and sports turf industries (Hanna et al., 2013).

Bermudagrasses are the primary warm-season turfgrass used on lawns, roadsides, parks, school grounds, athletic fields, golf courses, and anywhere a short, dense turf is desired

(Christians, 1998). Of the eight classified species of bermudagrass, only *C. dactylon* is widely distributed around the world (Mitich, 1989). Due to the dry climate under which it has evolved, bermudagrasses are deep rooted plants with low evapotranspiration rates, extensive lateral stem development, and superior drought tolerance (Beard, 2012). Bermudagrass spreads by rhizomes and stolons, and due to its growth habit, has a tendency to form a heavy thatch or mat layer (Wise, 1961). The root system develops by sprouting new fibrous roots from the nodes of either the stolons or rhizomes, which allows the plant to establish and spread rapidly (Steffurad, 1963). Although generally not very shade or cold tolerant, bermudagrass is a popular choice for a range of purposes in tropical to subtropical regions due to its ability to survive extended drought conditions and its performance under low-maintenance conditions (Christians, 1998).

One contributing factor to the superior bermudagrass performance in drier climates is these grasses possess a C_4 carbon metabolism. Unlike their C_3 relatives, C_4 plants do not undergo the costly process of photorespiration, which is a major disadvantage for C_3 plants in conditions such as high heat and drought. Due to their more efficient use of CO_2, C_4 plants can maintain a normal photosynthetic rate with smaller stomatal openings which increases their water use efficiency and reduces water loss (Raven et al., 2005).

Water Relations and Drought

Water Movement in Plants:

Water movement in plants begins in the soil and forms a continuum between the soil, the plant, and the atmosphere (Sheffer, 1982). Water in the soil enters the root through the microscopic cells called root hairs that increase the surface area of the root. Once absorbed by the root hairs, water flows across the cortical tissue via symplastic, transmembrane, and

apoplastic flow until it reaches the Casparian strip of the endodermis. At that point, the apoplastic flow is blocked and water is forced to cross the endodermis, and into the vascular tissue via the symplast (Taiz and Zeiger, 2002). Within the xylem, the main driving force for water movement in plants is evaporative loss of water to the atmosphere through the stomata (transpiration). Not only is transpiration an inevitable consequence to photosynthesis, water evaporation from the mesophyll cells also cools the leaf preventing the temperature from rising quickly to lethal levels (Lambers et al., 2008). The loss of water through the stomata lowers the water potential in the leaf cells which pulls a continuous column of water up through the entire length of xylem from the root to replace water that was lost to transpiration (Smith et al., 2010). This upward bulk flow of water is referred to as the cohesion-tension theory. It relies on the negative hydrostatic pressure within the xylem and the strong cohesive/adhesive properties of water which keeps the molecules together, as well as to the walls of the xylem as the column gets pulled up to the leaves from the roots. Water loss within the plant decreases the water potential at the roots and in turn, increases their capacity to uptake water from the soil. This results in a gradient of water potential from the leaves to the surface of the roots (Raven et al., 2005). During periods of water stress, plants are able to regulate their stomatal apertures by triggering the guard cells to lose turgor and close. This helps the plant avoid desiccation by slowing the loss of water through the leaves in order to conserve water during periods of drought (Lambers et al., 2008).

Drought in Plants

The term drought is most often used in meteorology to describe an abnormally long time period without significant rainfall. In plants, drought stress occurs when water uptake through

the roots becomes difficult, or when transpiration rates become very high (Reddy et al., 2004). Drought stress is characterized by reductions in plant growth, leaf water content, nutrient uptake, in addition to impaired light harvesting and carbon fixation ability (Farooq et al., 2012). Plants have adapted three drought resistant strategies to survive under a limited supply of water which include drought escape, drought tolerance, and drought avoidance (Levitt, 1980). Drought escape is the ability of a plant to survive drought stress by entering dormancy or by completing its life cycle. Drought avoidance is the ability of a plant to maintain growth during drought by reducing water loss through the leaves, or by increasing water uptake in the soil. Drought tolerance is the ability of the plant to maintain metabolic processes even under water limiting conditions (Huang, 2008). There exists a significant degree of genetic variability in drought resistance within and among most warm and cool season turfgrass species, including bermudagrass (Huang et al., 2014). The three drought resistant strategies are not mutually exclusive, and the same plant species may possess more than one strategy to adapt to drought stress (Nilsen and Orcutt, 1996).

Rooting and Drought

Roots are vital to plants ability to adapt to drought; and evidence suggests that it is the quality and not necessarily the quantity of roots that determine the most efficient strategy for extracting water from soil (Farooq et al., 2009). It is not uncommon for water to be more available at deeper soil depths while soil drying occurs at the surface. The importance of deep and prolific rooting is related to continued growth by providing water and nutrients to the plant even when part of the root system is under drying conditions, thereby contributing to drought resistance (Huang, 2008). Keeley and Koski (2002) reported drought tolerant cultivars of

Kentucky bluegrass (*Poa pratensis* L.) had greater percentages of their root systems distributed in deeper soil layers compared to shallow rooted cultivars. Plant species that have more extensive root systems are able to better resist desiccation during drought due to increased water uptake ability. Plants have evolved several different methods to harvest water from large soil volumes such as increased rooting depth, surface area, density, and mass (McCann and Huang, 2008a; McCann and Huang, 2008b).

Rooting Studies in Turfgrass

Several studies have been conducted in turfgrass that have related drought resistance to rooting characteristics. Karcher et al. (2008) reported that tall fescue (*Festuca arundinacea* Schreb.) varieties that had a greater root to shoot ratio were more drought resistant than varieties with lower root to shoot ratios. According to Marcum et al. (1995), among 25 cultivars of zoysiagrass (*Zoysia spp.*), those with greater rooting depth and root weight at deeper soil depths displayed superior green turfgrass cover under three levels of water deficit. Qian et al. (1997) reported that the shallow rooted zoysiagrass cultivar, 'Meyer', had inferior drought avoidance compared to other cultivars with more prolific roots as evidenced by more leaf wilting under drought conditions. In the same study, it was shown that tall fescue plants were able to harvest up to 50% more water than zoysiagrass at a soil depth of 90 cm as a result of greater root length and root length density. In creeping bentgrass (*Agrostis stolonifera* L.), McCann and Huang (2008b) reported that genetic variation for drought tolerance existed among cultivars. The authors concluded that creeping bentgrass plants that were able to maintain better quality under drought were typically characterized by increased root production and elongation (McCann and Huang, 2008b). In a study by Suplick-Ploense and Qian (2005), 'Reville' hybrid bluegrass [*Poa*

pratensis L. × *Poa arachnifera* Torr.] had greater root length density and root mass than 'Bensun' Kentucky bluegrass leading to Reville maintaining higher turfgrass quality under a prolonged drought. Under limited soil moisture, higher turfgrass quality and less leaf firing was observed in tall fescue varieties that had increased root growth at deeper soil depths compared to Kentucky bluegrass (Ervin and Koski, 1998).

Oxidative Stress and Carotenoids

Formation of ROS

During photosynthesis, quanta of light energy are absorbed by the photosynthetic antenna pigments of photosystem II (PSII) in the chloroplast membranes, resulting in the formation of an excited state of the P680 chlorophyll molecule (Inze and Van Montagu, 2002). The excitation energy then moves through the light harvesting complex antenna to the reaction center chlorophyll molecules which leads to a charge-separation event in PSII (Inze and Van Montagu, 2002; Smith et al., 2010). The electron generated by the separation is passed along to plastoquinone on the D1 protein, and along the electron transport chain to the P700 molecule which is reduced in order to produce NADPH required for carbon assimilation by the Calvin cycle. P680 is then reduced by the transfer of electrons from the water splitting complex of PSII, producing oxygen.

Reactive oxygen species (ROS) are formed with the reduction of molecular oxygen into the superoxide radical, (O_2^-), which is highly reactive and can oxidize amino acids and lipids, causing damage to the cell. Further reduction of this superoxide radical will result in the formation of two other species, hydrogen peroxide (H_2O_2), and the most toxic, hydroxyl radical ($HO^\bullet$). The hydroxyl radical has a high affinity for all biological molecules and can cause severe

cellular damage (Smith et al., 2010). Under normal circumstances, most sunlight absorbed by the plant is used for photosynthesis. However, under stressful conditions such as high light or drought, excitation energy will accumulate when the rate of photons absorbed exceeds the rate used for photosynthesis (Demmig-Adams, 1990). During drought, stomatal closure induced by abscisic acid signaling can result in the over-reduction of the photosynthetic apparatus. The plant continues to produce excited chlorophyll molecules, but carbon assimilation rates decline due to reduced levels of carbon dioxide available to fuel the Calvin cycle reactions as a result of decreased gas exchange through the stomata (Inze and Van Montagu, 2002). It is the resulting buildup of excess of excitation energy among chlorophyll molecules that leads to the formation of triplet state chlorophyll, which can react with molecular oxygen present within the cells to form ROS (Demmig-Adams, 1990). Oxidative stress can occur when the scavenging of ROS falls below the production of ROS (Fu and Huang, 2001) in situations such as drought, high light, and high or low temperatures. In order to prevent death, plants must have effective ROS scavenging mechanisms, including enzymatic and non-enzymatic antioxidants (Latowski et al., 2011). Through the use of different antioxidants and ROS scavengers, plants can enhance resistance to stresses such as drought (Vinocur and Altman, 2005).

Antioxidant Systems

Antioxidant defense mechanisms are a plant's main defense against the destructive characteristics of ROS. The antioxidant defense systems of plants consist of both enzymatic and non-enzymatic components, and when a plant is under a stress such as drought, high activities of both types of systems are important (Farooq et al., 2009). The enzymatic antioxidant defense system consists of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX),

glutathione reductase (GR), and glutathione peroxidase. The non-enzymatic scavenging systems consist of ascorbate and glutathione, in addition to tocopherol, flavonoids, alkaloids, and carotenoids (Apel and Hirt, 2004).

There has been more turfgrass-related drought research that has investigated the activity of enzymatic antioxidant systems rather than the non-enzymatic systems. Fu and Huang (2001), found that increased SOD activity played a key role in protecting both Kentucky bluegrass, and tall fescue from superoxide radicals for 40 days while withholding irrigation. Similarly, Lu and Chen et al. (2009) found that somaclonal variants of 'TifEagle' hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *Cynodon transvaalensis* Burtt-Davy] had increased CAT activities resulting in increased drought resistance after 8 days of stress. Hatamzadeh and Molaahmad Nalousi et al. (2014) reported that activity of SOD and APX was increased after 40 days of drought in the leaves of creeping bentgrass (*Agrostis stolonifera* var *palustris*) and tall fescue following exogenous nitric oxide application resulting in alleviation to drought stress.

Non-enzymatic Antioxidant System – the Xanthophylls

In the lowest excited state, chlorophyll has three pathways to dissipate absorbed light energy: (i) the energy is used for the photochemistry involved in converting CO_2 into sugars termed, photochemical quenching (qP), (ii) the energy can be re-emitted as a lower energy photon in a process known as fluorescence, and (iii) the energy can be dissipated as heat through non-photochemical quenching (NPQ) (Demmig-Adams and Adams III, 1996a; Taiz and Zeiger, 2002). These three processes function together, and any increase or decrease in activity in one process is reflected in the other two. For example, if damage occurs to the photosystem, qP will decrease as the fluorescence and NPQ parameters increase (Demmig-Adams and Adams III,

1996b; McElroy and Kopsell, 2009). Because fluorescence is easily measured as a function of photochemical efficiency (Fv/Fm) using a fluorimeter device, it is often used as an indicator of plant drought stress in turfgrass research (DaCosta and Huang, 2007; Du et al., 2009; Hu et al., 2009; Zhao et al., 2011). F_v/F_m is determined by subtracting minimal fluorescence parameter (F_0) from the maximum level of fluorescence (F_m) to yield the variable fluorescence parameter, F_v. The ratio of F_v to F_m is indicative of the quantum yield of PSII photochemistry, and is directly proportional to CO_2 assimilation (Genty et al., 1989).

Non-photochemical quenching is a term often used to describe the quenching of singlet state chlorophyll, and the dissipation of excess energy as heat (Müller et al., 2001). NPQ is one way plants can protect the photosynthetic machinery against over excitation, and involves three carotenoid antioxidant pigments called xanthophylls. Xanthophylls can function in two ways, either as accessory pigments for light harvesting in PSII, or in NPQ where excess energy is converted to heat (Taiz and Zeiger, 2002). Violaxanthin, antheraxanthin, and zeaxanthin are the three pigments that make up what is known as the xanthophyll cycle, and are present in the thylakoid membranes of all higher plants (Demmig-Adams et al., 1996). In high light conditions, violaxanthin is de-epoxidized once to form antheraxanthin, and then again to form zeaxanthin. In low light conditions, the reverse occurs as zeaxanthin is epoxidized first to the intermediate molecule, antheraxanthin, and then again back to violaxanthin (Lambers, 1998).

Non-enzymatic Photoprotection Studies

There has been a great deal of turfgrass drought research evaluating plant characteristics such as water use, root characteristics, photosynthetic protein expression, hormone accumulation, and enzymatic antioxidant levels. However, very little research has been put forth in turfgrasses

investigating the non-enzymatic photoprotection systems such as the xanthophyll cycle in response to water deficit.

A study conducted by McElroy (2006), examined the response of xanthophyll cycle pigments in creeping bentgrass when subject to high and low levels of irradiance. Xanthophyll cycle pigment concentrations for 'Crenshaw' creeping bentgrass were found to accumulate to high levels when grown under high irradiance, which could potentially contribute to better performance under other stressful conditions (McElroy et al., 2006). Other studies have examined xanthophyll cycle pigment response to herbicide treatments (Brosnan et al., 2011). However, there have been no studies performed on turfgrass that have examined xanthophyll cycle pigment activity in response to drought (Asada, 2000).

Experiments have been carried out in a variety of non-turfgrass plant species that have investigated the responses of non-enzymatic antioxidants such as α-tocopherol and carotenoids to abiotic stresses like drought. Studies that overexpressed genes that encode for β-carotene hydroxylase enzymes (enzymes used in the zeaxanthin biosynthetic pathway) have been shown to increase xanthophyll cycle pigments and improve stress tolerance compared to wild types in *Arabidopsis*, tobacco (*Nicotiana tabacum*), and rice (*Oryza sativa*) (Davison et al., 2002; Du et al., 2010; Wu et al., 2015). In experiments with rosemary (*Rosmarinus officinalis* L.), lemon balm (*Melissa officinalis* L.), lyreleaf sage (*Salvia lyrata* L.), and Chinese dwarf cherry seedlings (*Cerealis humilis*), increased concentrations of tocopherols, and xanthophyll pigments have led to enhanced photoprotection and drought stress tolerance (Munné-Bosch and Alegre, 2000a; Munné-Bosch and Alegre, 2000b; Munné-Bosch and Cela, 2006; Song et al., 2011).

Justification and Objectives

Water use and conservation have become increasingly important across the United States. Continuing to identify drought resistant mechanisms in turfgrasses will help aid breeders in the development of new varieties that can better survive during extended periods of drought. Reducing water input requirements is one way that the turfgrass industry can help to alleviate the need for irrigation to maintain quality turfgrass. Recently released cultivars of hybrid bermudagrass such as 'TifTuf' and 'Latitude 36' have demonstrated improved resistance to stresses like drought and cold temperatures compared to commonly used cultivars like 'Tifway'. Differences in rooting characteristics among several species of turfgrasses have been demonstrated to have an effect on drought resistance. Therefore, the first objective of the research presented herein is to determine if differences in root morphology among three cultivars of hybrid bermudagrass plays a role in drought resistance.

Enzymatic antioxidant systems are another means by which plants can defend against cellular damage resulting from drought. Several experiments conducted in turfgrass have shown that increased enzymatic antioxidants (SOD, APX, GPX, etc.) can help improve drought tolerance. However, despite their ability to improve drought stress tolerance, the non-enzymatic antioxidants such as β-carotene and the xanthophylls have received very little attention in turfgrass research. It is unknown why the non-enzymatic antioxidants are less studied, but one potential reason could be due to the fact that they are not as quickly and cheaply quantified compared to enzymatic antioxidants (McElroy and Kopsell, 2009). Data collected in several plant species have indicated increased contents of carotenoids are important for stress tolerance through NPQ and ROS scavenging. Plants with the ability to scavenge and/or control the level of ROS may be useful in the future to withstand drought (Gill and Tuteja, 2010). This warrants

more concerted efforts for turfgrass researchers to explore the responses of these pigments to water deficit and what roles they may play in drought tolerance. Hence, the second objective of this research is to evaluate the response of antioxidant pigments to drought, and to determine if differences in antioxidant concentrations among cultivars may contribute to drought tolerance.

CHAPTER II - ROOTING CHARACTERISTICS OF THREE HYBRID BERMUDAGRASS CULTIVARS EXPOSED TO DROUGHT

Abstract

Drought stress is one of the most detrimental abiotic stresses that severely limits the growth and productivity of all plants. Recently released cultivars of hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *Cynodon transvaalensis* Burtt-Davy] have demonstrated potentially improved tolerance to various stresses, but the responses of these cultivars to drought are not well understood. This study was conducted to evaluate the relationship of rooting characteristics to the drought resistance of three hybrid bermudagrass cultivars. Plants were grown in 45-cm tall lysimeters and arranged in a three replication, completely randomized design. Lysimeters were subjected to 28 d consecutive drought stress under field conditions in the summer of 2015. At the end of the drought period, roots were washed free of soil and divided into three 15-cm sections. Evaluations included turfgrass quality, root dry mass and length, in addition to assessments of total root length, root length density, root volume, and root surface area using WinRHIZO software. Results indicated that 'TifTuf' maintained higher turfgrass quality than 'Latitude 36' and 'Tifway' 8 to 20 d after withholding water, indicating superior drought resistance. Improved drought resistance of TifTuf was characterized by producing 70 and 55% more dry root weight at the 15 – 30 cm soil depth, and 160 and 143% more root dry weight at the 30 – 45 cm soil depth than Latitude 36 and Tifway, respectively. TifTuf also produced significantly greater root surface area and volume at the 30 – 45 cm soil depth. Our findings suggest that TifTuf likely utilizes drought avoidance characteristics to maintain superior turfgrass quality under drought conditions.

Introduction

As water increasingly becomes a limiting factor on Earth, the importance of more drought resistant plants has intensified. Recreational and residential turfgrass areas are often viewed as a strain on the water supply due to their inherent need for supplemental irrigation during periods of limited water. As a result, local authorities have begun to restrict the use of turfgrasses in urban areas (SNWA, 2014). When these restrictions are enacted, the ecological and economic benefits of turfgrass systems are overlooked, and their beneficial roles within the landscape are lost. In order to help turfgrasses retain their place as part of the modern landscape, the search for more drought resistant turfgrasses that require less water for survival should be among the utmost of priorities.

One strategy used by plants to survive drought is through drought avoidance (Levitt, 1980). Drought avoidance is often characterized by increasing uptake of water through the roots. Drought avoiding plants typically can survive short-term drought until all available soil water has been depleted (Huang, 2008). Plant species with more extensive root systems have the ability to better resist desiccation during drought conditions. Plants harvest water from large soil volumes by increasing root depth, surface area, density, and mass (Huang et al., 2014; McCann and Huang, 2008a). A robust root system has the ability to support plants during drought due to its ability to harvest more water from the soil (Jaleel et al., 2009). Plants with extensive root systems are commonly sought for selection in food crops due to their ability to acquire more water (Subbarao et al., 1995). Several researchers have associated turfgrass rooting characteristics with drought avoidance (Karcher et al., 2008; Marcum et al., 1995; McCann and Huang, 2008b; Qian et al., 1997; Suplick-Ploense and Qian, 2005).

Karcher et al. (2008) reported that tall fescue (*Festuca arundinacea* L.) varieties with a greater root to shoot ratio had superior drought avoidance compared to varieties with low root to shoot ratios. According to Marcum et al. (1995) among 25 zoysiagrass (*Zoysia spp.*) cultivars, those with superior rooting depth and root weight at greater soil depths displayed superior drought avoidance. Qian et al. (1997) found that the shallow-rooted 'Meyer' zoysiagrass cultivar had inferior drought avoidance compared to other turfgrass species with more prolific roots, which exhibited less leaf wilting than Meyer under drought conditions. In the same study, it was reported that tall fescue plants were capable of harvesting 50% more water at a soil depth of 90 cm than zoysiagrass as a result of greater total root length and root length density at deeper soil depths (Qian et al., 1997). In a study of creeping bentgrass (*Agrostis stolonifera* L.), McCann and Huang (2008b) observed genetic variation among cultivars for drought avoidance, with cultivars able to maintain better quality under drought conditions typically having increased root elongation and production. In a field and glasshouse study, Suplick-Ploense and Qian (2005) concluded that 'Reville' hybrid bluegrass [*Poa pratensis* L. *x Poa arachnifera* Torr.] had greater root length density and root mass than 'Bensun' Kentucky bluegrass (*Poa pratensis* L.), which enabled Reville to maintain higher turfgrass quality than Bensun during prolonged drought. Under limited soil moisture, higher turfgrass quality and less leaf firing was found in tall fescue varieties having increased root growth with which to extract water from deeper soil layers compared to Kentucky bluegrass (Ervin and Koski, 1998).

A recently released cultivar of hybrid bermudagrass [*Cynodon dactylon* L. *x Cynodon transvaalensis* Burtt-Davy cv. 'TifTuf'] may have the ability to better withstand water deficit compared to other bermudagrass varieties (Schwartz, 2014). Due to its sustained quality and green color under drought stress, TifTuf (experimental ID: 'DT-1') was selected from a trial of

90 other hybrid bermdagrass experimental lines under a rainout shelter in Tifton, GA. TifTuf has demonstrated the potential to maintain quality longer and recover from drought stress in less time than other cultivars of hybrid bermudagrass commonly used in the southeastern United States such as 'Tifway' and 'Latitude 36' (Schwartz, 2014); however, mechanisms explaining this response are not well defined.

Increasing knowledge about the enhanced drought resistance of new cultivars like TifTuf can help breeders select more drought resistant turfgrasses. Identifying new turfgrasses with improved drought resistance may also help reduce the need for supplemental irrigation for turfgrass areas. Using modern digital imaging methods to analyze root architecture can help yield more accurate results compared to the older and more subjective approaches to plant root analysis. Therefore, the objectives of this study were to evaluate three commercially available hybrid bermudagrass cultivars to determine how, if at all, drought resistance, root architecture, and water use differ among them.

Materials and Methods

Plant Culture

The three hybrid bermudagrass cultivars chosen for this study were TifTuf, Latitude 36, and Tifway. TifTuf and Latitude 36 were chosen because they are among the newest commercially available hybrid bermudagrasses for turfgrass use in the U.S. In addition, their relative drought resistance has not been thoroughly researched to date. Tifway has been a standard variety used in a range of turfgrass experiments across the U.S. and was included as a check cultivar for this research. Round plugs 5 cm in diameter x 5 cm in depth were taken from field plots established on Sequatchie silt loam soil (fine-loamy, siliceous, Typic Paleudults) at

the East Tennessee Research and Education Center (ETREC) on 15 Sept 2014 using a manually operated sod plugger. Plugs were transferred into 5 cm deep plastic trays filled with a peat-based potting mix (Pro-Mix Bx, Fafard, Inc., Quakertown, PA). Trays were taken into a glasshouse to allow the plugs to establish a root system and to increase aboveground biomass. Plants were maintained between 21 °C nighttime and 35 °C daytime temperatures with an 18 h photoperiod. Trays were fertilized once every 2 weeks through a syringe with a 400 ml solution of water soluble fertilizer (UltraSol 20 N-20 P_2O_5-20 K_2O, SQM Co., Atlanta, GA) at a rate of 25 kg N ha^{-1}. All plants were hand-trimmed using scissors two to three times per week to a height of 10 cm while establishing in the trays, and clippings were returned. Supplemental irrigation was supplied via an overhead irrigation system set to run four cycles per d for 15 min each to prevent desiccation.

Plant Propagation

Lysimeters were constructed with 10.3-cm (inside diameter) PVC sewer pipe. The pipe was cut into 45-cm sections to allow for adequate root growth. Small pieces of landscape fabric were cut into roughly 15 x 15 cm sections, and placed over one end of the pipe. The edges of the fabric were folded over the sides of the pipe and pulled tightly to cover the entire opening. Outdoor-grade duct tape was used to fasten the cloth to the outside of pipe, creating a water permeable barrier to allow for drainage and was strong enough to hold the growing media in place (Figure 1). Note: all figures referenced hereafter are displayed in the appendix section at the end of this document. Lysimeters were filled with dry 100% silica sand (Short Mountain Silica, Mooresburg, TN) graded to the United States Golf Association specification particle size

(Hummel, 1993) to serve as the growing media. All lysimeters were filled with 6.18 kg of dry sand in order to obtain a relatively uniform root zone bulk density of 1.57 g cm^{-3} (Figure 2).

Vegetative propagation of plant material into the lysimeters began on 26 May 2015. Plants of three tillers each were removed from the trays and washed free of any soil/potting mix left adhering to the roots. Plants were trimmed to a uniform height of 8 cm, and roots were trimmed to a length of 1-cm before being planted into the lysimeters. Eight evenly spaced plants were placed in approximately 7-cm deep pre-bored holes in the sand of each lysimeter (Figure 3). Plants were fertilized with a controlled release granular fertilizer (Greenlinks 24 N- 4 P_2O_5 - 12 K_2O, Tyler Enterprises, Morris, IL) one week after propagation at a rate of 50 kg N ha^{-1}.

Plants were allowed to establish in the lysimeters for 5 weeks under the same glasshouse growing conditions as previously described, except irrigation was increased to eight 15 min cycles per d. After 5 weeks, plants were transferred to ETREC to complete establishment in the field environment which this experiment was to be conducted. Lysimeters were supplied water from an in-ground system set to deliver 15 min of water 8 times per d. Upon being placed in the field, all lysimeters were fertilized again at a rate of 50 kg N ha^{-1} with the same controlled release fertilizer used previously. Due to the low nutrient holding capacity of sand, a diluted rate (5 kg N ha^{-1}) of the same 20 N – 20 P_2O_5 – 20 K_2O fertilizer was applied to the foliage 5 times per week using a hand spray bottle at an application volume of 2.5 ml per lysimeter. This was to help offset the loss of soil-applied nutrients due to leaching through the sand-based soil profile. All plants were hand trimmed with scissors two to three times per week at a height of 3.8 cm, and clippings were returned.

Rainout Box

Two small rainout boxes (1.2m L x 0.9m W x 0.5m H) were built to the same height as the lysimeters using 1.3-cm thick plywood to create the walls. A lid was constructed out of clear acrylic roofing panels that were fastened to a hinged frame and then attached to one of the walls of the box (Figure 4). The lid could be opened and closed manually during periods of rain. The purpose of the lid was to protect the plants inside from rain, while simultaneously allowing light to enter during periods when the lid was closed. To help keep the sides of the rainout box insulated from excess heating due to direct exposure to sun radiation, a double reflective radiant barrier (Reflectix, Markleville, IN) was stapled to both the inside and outside faces of the walls of each box (Figure 4).

Experimental Design

The study began 10 weeks after initial propagation when turfgrass canopy coverage in all lysimeters reached 100% based on visual assessment. A total of 72 plants were arranged within each rainout box in a completely randomized design with three replications. The experiment was split into two locations; therefore, a split-plot treatment design was applied to the analysis to test for treatment interactions by location, using location as the whole plot factor and cultivar as the sub-plot factor. One box was placed at ETREC, and the other was positioned at the University of Tennessee's Institute of Agriculture (UTIA). At ETREC, the rainout box was placed in an area of full sun and wind exposure amongst plots at the University of Tennessee's Center for Athletic Field Research (35°54'05.2" N Lat., 83°57'34.5" W Long.). The rainout box at UTIA was placed in a location surrounded by nursery plant greenhouses (35° 56' 46.3" N Lat., 83° 56' 18.6" W Long.), which provided greater protection from wind in addition to shade in the early

morning/late afternoon. A weather station (WatchDog 1450 series, Spectrum Technologies, Auroroa, IL) was attached to each rainout box to record the average daytime temperature and humidity data in 1 h increments each d between 0800 and 2000 (Figure 5).

Drought Conditions

Drought was imposed by withholding all rainfall and irrigation for a period of 28 d beginning on 3 Aug 2015 at ETREC and 5 Aug 2015 at UTIA. To ensure no moisture was provided to the plants during the drought cycle, the lid was placed over the plants during periods of rain, as well as at night when researchers were not present.

Data Collection

Measurements of soil water content (SWC) were taken at the beginning of each experiment and again at 28 d after withholding water (DAWW), in order to estimate water use. Before drought began, soil moisture in each lysimeter was brought to field capacity by completely submerging the lysimeter in water. Air bubbles observed rising to the surface from the top of the lysimeter indicated that air space within the rootzone was being infiltrated with water, forcing the air upward out of the pores of the sand. After a period of approximately 15 min, air bubbles were no longer visibly rising from the lysimeters, and at that point the rootzone was considered to be fully saturated. Lysimeters were then removed from the water and placed on a porous gravel surface to drain freely. After water was no longer seen dripping from the bottom of the lysimeter (approximately 30 min), they were weighed using an electronic balance to obtain the water weight at field capacity. At 28 DAWW, the lysimeters were weighed again to determine the total SWC lost during drought based on the difference in mass. The calculation for SWC was based on the known dry mass of the entire sand column within the lysimeter of

6.18 kg plus the empty lysimeter weight subtracted from the total wet mass of each lysimeter using the following equations:

Eq. 1: water mass = lysimeter mass – (6.18 + mass of empty lysimeter)

Eq. 2: %SWC = (water mass) / (6.18) × 100

Visual turfgrass quality (TQ) was evaluated at 4 d intervals over the drought cycle. Ratings were assessed using a 1 to 9 scale (1 = poorest quality turf, 6 = minimum acceptable quality, 9 = ideal turf), according to Morris and Shearman (1998). Three lysimeters of each variety were rated on each sampling date for TQ. The plants within each rating date were subsequently removed from the box after TQ was evaluated in order to be used for aboveground tissue analysis in a concurrent study presented in Chapter 3. Turf quality data were analyzed with a non-linear regression sigmoidal variable slope model using Prism v. 6.05 software (GraphPad Prism, San Diego, CA) similar to Karcher et al. (2008). The model was used to predict the number of days necessary to produce a drought response of any amount relative to the maximum possible value TQ (TQ_X), where: $Y = (100)/((1+10^{(DaysX-DAWW)}) \times Slope))$. In this equation, Y represented the measured TQ value, 100 indicated the maximum TQ (based on percent), $Days_X$ was the number of estimated days to produce a response of the maximum potential TQ, and the slope variable showed the rate of change in TQ. A sum of squares F-test was used to compare the differences in model parameter estimates between global (shared $Days_X$) verses individual ($Days_X$ for each cultivar), and if the test was significant ($P \leq 0.05$), then cultivar effects were determined to be significant (Steinke et al., 2011). Parameter estimates were then used to calculate 95% confident intervals for the number of DAWW until each cultivar

reached TQ = 6 in response to drought. Cultivar responses were considered significantly different if their confidence intervals did not overlap (Karcher et al., 2008).

At the end of the 28 d drought period, entire plants were removed from the lysimeters and the roots were washed free of sand with water using a hose-end nozzle sprayer. The roots were separated from the aboveground tissue using scissors to cut away the roots below the crown of the plant. Whole plant root length (RL) was then measured with a ruler from the top of the root system to the tip of the longest root. Following this, roots were cut with hand shears into three equal sections at the 0 to 15, 15 to 30, and 30 to 45 cm lengths. Root sections were analyzed for total root length (TRL), surface area (SA), root volume (RV), average diameter (AD), and root length density (RLD) using WinRHIZO image analysis software (v. 2008b Regent Instruments, Quebec, Canada). To make separation of roots easier, cleaned root samples were suspended in a thin layer of water inside of a 20 x 25 cm clear acrylic scan tray prior to image acquisition. This allowed for easier separation of finer roots on the scan tray, thus allowing a more detailed image of root architecture. Roots were gently spread apart in the trays by hand, and a scanner (V700, Epson America, Long Beach, CA) was used to obtain scanned images of the roots at 600 dpi resolution. Roots in the 0 to 15 cm section were detached from the rhizomes using tweezers prior to scanning in order to obtain rhizome dry weight (RhDW). After image analysis was complete, root sections and rhizomes were dried in an oven at 72 °C for 48 h to obtain measurements of root dry weight (DW) and RhDW. All root analysis data were subjected to mixed model analysis of variance using the GLIMMIX procedure in SAS 9.4 (SAS institute, Cary, NC), and means were separated using Fisher's protected LSD at the $P \leq 0.05$ level of probability.

Results

All data were subjected to a square root transformation to correct for issues of unequal variance. Interpretations of backtransformed means were similar to the untransformed (original) means; therefore, the untransformed means are presented for clarity. No treatment-by-location interactions were detected by the analysis of variance for any of the dependent variables measured. Therefore, means presented hereafter were pooled across both locations.

Visual TQ declined differently for each cultivar as drought progressed (Figure 6). Turfgrass quality ratings were higher for TifTuf compared to Latitude 36 and Tifway between 8 and 20 DAWW. TifTuf was able to maintain an acceptable TQ level of 6 or above through 17 DAWW; this was significantly longer than Latitude 36 or Tifway ($P < 0.001$). The number of days until TQ levels were no longer acceptable for Latitude 36 and Tifway was 9.0 and 6.0 DAWW, respectively (Table 4). After 24 d of drought stress, TQ was similar among all three cultivars.

A significant day-by-cultivar interaction occurred for SWC between 0 and 28 DAWW (Figure 7). All treatments were initiated with approximately 15% SWC (i.e., field capacity) at the beginning of the study. After 28 DAWW, SWC for TifTuf dropped to 2.5%, which was significantly less than that of the Latitude 36 and Tifway. On the basis of total water volume lost to evapotranspiration between 0 and 28 DAWW, TifTuf lost 848 ml while water loss for Latitude 36 and Tifway was 637 and 698 ml, respectively.

Main effect differences were detected ($P < 0.05$) among cultivars for rhizome dry mass in addition to root dry mass at the 15 – 30 and 30 – 45 cm root zone depths (Table 1), (all tables referenced hereafter are displayed in the appendix section at the end of this document). Root DW did not differ between any cultivars at the 0 – 15 cm depth; however, RHDW for the TifTuf

cultivar was greater compared to Latitude 36 but similar to Tifway. Average root DW at the 15 – 30 cm depth was significantly greater for TifTuf than that of the other two cultivars. Average root DW was also greater for TifTuf at the 30 – 45 cm depth compared to the other two cultivars, which were not different from one another. The total DW for the entire root system (0 – 45 cm) differed among the three cultivars. TifTuf produced a greater total DW than Latitude 36 and Tifway. Differences between RL for TifTuf, Latitude 36, and Tifway were not statistically different.

Differences in root SA (cm^2) and RV (cm^3) only occurred at the 30 – 45 cm root zone depth (Table 2). At the 30 – 45 cm depth, SA for TifTuf was 6.4x and 4.2x greater than the Latitude 36 and Tifway cultivars. Root volume for TifTuf at this depth was 7.5x and 4.5x greater than the Latitude 36 and Tifway cultivars. Main effect differences for cultivar were identified for the entire root system profile (0 – 45 cm depth) for SA and RV (Table 2). Mean total root SA for TifTuf was not statistically different from Tifway; however, TifTuf produced greater total root SA than Latitude 36. Measurements of total RV revealed that TifTuf had significantly more volume than Tifway and Latitude 36. Additionally, no significant differences were detected among cultivars for TRL, SA, AD, or RV within the 0 – 15 cm soil depth.

There were no differences detected for RLD between any cultivars at any of the three root zone depths; however, it may be of interest to note that RLD of TifTuf was 1.6 times greater ($P = 0.11$) at the 15 – 30 depth and 5.4 times greater ($P = 0.06$) at the 30 – 45 cm depth than the more drought sensitive cultivar, Latitude 36. Likewise, the RLD of TifTuf measured for the entire root system from 0 – 45 cm was 1.3 times greater than that of Latitude 36; however, this was only statistically significant at the $P = 0.06$ level of probability, as well. More observations may be needed to fully determine the significance of these measurements.

Discussion

Results indicate that TQ differed among cultivars, and that the change in TQ differed among cultivars over time. Turf quality of TifTuf remained above the minimum acceptable level for a longer period of time compared to the other cultivars, indicating improved drought resistance. Soil water extraction characteristics differed among cultivars. The TifTuf cultivar having higher root weight, SA, and RV at deeper soil depths utilized more water over the 28 d drought period.

Abraham et al. (2008) reported that improved TQ of hybrid bluegrass was related to more extensive root growth, particularly at a deeper soil depths. Although mean RL for TifTuf was approximately 1.2 times greater than Latitude 36 and Tifway, RL did not statistically differ among varieties. This suggests that deeper roots did not necessarily result in better performance under drought in the present study. Because of the high degree of variability among cultivars, evaluations of RL alone may not be a good indication of a drought resistant turfgrass. Compared to Latitude 36 and Tifway, TifTuf produced more total root dry root mass throughout the soil profile, in large part due to greater root dry mass production at the 15 – 30 and 30 – 45 cm soil depths. Root mass deeper in the soil profiles may be more related to the drought resistance of TifTuf, similar to reports by Marcum et al. (1995) and Huang et al. (1997). In this study, TifTuf produced 41% of its total root biomass from 15 – 45 cm compared to only 22 and 26% for Latitude 36 and Tifway. These data suggest that the superior TQ ratings of TifTuf under drought stress may be linked to this characteristic.

The increased root production for TifTuf at deeper soil depths is perhaps best reflected in the RV and SA analyses shown in Table 2. At the deepest soil depth, TifTuf produced 7.5 and 4.4 times more RV than Latitude 36 and Tifway. Total (0 – 45 cm soil depth) RV was also

greatest for the TifTuf cultivar; however, since RV was not significantly different among cultivars from 0 – 30 cm, the largest contributing factor to this difference was most likely due to the higher RV in the 30 – 45 cm soil depth.

Root SA in the 30 – 45 cm soil depth was greater for TifTuf compared to the other two cultivars. This is most likely a direct result of having more roots present in general at this depth. Nonetheless, water uptake in plants is dependent on having a large root surface area to allow for maximum water absorption (Raven et al., 2005). Li et al. (2015) reported that SA was linked to better drought performance in hybrid maize (*Zea mays* L.) seedlings under water stress. Hence, the increased root SA for TifTuf may have contributed to its ability to harvest more water particularly from the deeper soil depth, which could have affected its ability to maintain higher quality under stress.

Soil water content decreased more for TifTuf compared to Latitude 36 and Tifway. Between 0 and 28 DAWW, TifTuf lost approximately 21% more water volume compared to Latitude 36 or Tifway. This is most likely due to the increased water uptake capacity of the TifTuf cultivar as a result of its more robust rooting characteristics at deeper depths within the soil profile. According to the report of Qian et al. (1997), superior rooting ability of tall fescue led to less leaf wilting in the field indicating that tall fescue is an excellent drought avoider. This however, was in comparison to turfgrasses of a different genus. As the data in this study suggests, TifTuf may also possess similar drought avoidance characteristics due to its rooting abilities, and these characteristics were different between plants of the same variety. In comparison to the other cultivars used in this study, TifTuf was able to maintain better quality, which could be due to its increased rooting characteristics at deeper soil depths.

Rhizome dry mass for TifTuf was significantly greater than Latitude 36, but not for Tifway. In a study conducted on 18 bermudagrass genotypes from Australia, drought resistant grasses were able to extract more water from the soil due to increased rhizome production rather than RLD or rooting depth (Zhou et al., 2013). Additionally, RLD in the same study was determined not to be correlated to drought resistance (Zhou et al., 2013). Although differences in RLD were considerably large in our study (27.85 to 150.46 $m \cdot m^{-3}$ at the 30 – 45 cm depth), RLD measurements did not differ significantly among cultivars, indicating that this factor may have a limited role in drought resistance of hybrid bermudagrass. However, the high degree of variability in the root data and the relatively small number of samples used for our experiment may be another reason explaining the lack of significance differences in RLD among cultivars.

Interestingly, rhizome production differed among cultivars in this study, which was also reported by Zhou et al. (2013). According to Suzuki and Stuefer (1999), rhizomes can be used as a source/sink organ for nutrients and water which can then be supplied to other parts of the plant to maintain growth under adverse environmental conditions. It was hypothesized by Zhou et al. (2013) that more rhizomes may be able to supply roots with nutrients and water in order to maintain root function. There is considerable variation among bermudagrass varieties for rhizome production (Casler and Duncan, 2003); however, further investigation into turfgrass rhizome function is needed to fully understand the extent of their relationship to drought resistance.

In conclusion, TifTuf was able to maintain better TQ over a period of 28 days with no rain or irrigation. Rooting characteristics are most likely the reason for the improved drought resistance compared to other popular cultivars used like Latitude 36 or Tifway. In particular, TifTuf produced more root DW, RV, and SA at deeper soil depths compared to the Latitude 36

and Tifway cultivars. The increased rooting capability of TifTuf also led to an increase in its overall water use indicating that this particular cultivar may be able to better withstand drought stress due to avoidance characteristics. Since this study was carried out in manufactured sand-filled lysimeters, the experiment should be replicated under natural field conditions in order to confirm these results. As new drought resistant varieties like TifTuf are being developed, research should continue to focus on determining the means by which these grasses survive drought i.e., deeper rooting, water use efficiency, in addition to physiological parameters such as antioxidant defenses. Additionally, rhizome production as it relates to drought resistance should be further investigated for grasses like TifTuf to determine the allocation and transport of water/nutrients/carbohydrate these structures may facilitate during periods of stress. From the research presented herein, turfgrass breeders will be able to consider rooting characteristics such as weight, surface area, and volume at soil depths of 30-45 cm when making selections for the advancement of drought resistant species.

CHAPTER III - ANTIOXIDANT PIGMENT RESPONSES OF THREE HYBRID BERMUDAGRASS CULTIVARS EXPOSED TO DROUGHT

Abstract

Antioxidant defense systems are one mechanism plants use to defend against the negative effects of drought stress, particularly the production of reactive oxygen species (ROS). Previous turfgrass drought studies have demonstrated that enzymatic antioxidants can provide resistance to drought stress via ROS scavenging. However, information on the activity of non-enzymatic antioxidants, such as carotenoid pigments, in response to drought is lacking. This study was designed to compare the response of antioxidant pigments among three cultivars of hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy] with putative differences to drought stress. Plants were established in lysimeters and subjected to 28 consecutive d of drought stress under field conditions at two locations in East Tennessee by withholding all rain/irrigation during the summer of 2015. Data were collected at 4 d intervals throughout the study. Indications of drought stress tolerance were based on observations of turfgrass quality, percent green cover, and relative leaf water content. Chlorophyll and carotenoid pigments were extracted and measured using high performance liquid chromatography. The cultivar, 'TifTuf' displayed superior performance under drought stress conditions as indicated by better turfgrass quality and percent green cover compared to other cultivars at the mid to late stages of drought (between 8 and 20 days after withholding water). Concomitantly, TifTuf had increased concentrations of zeaxanthin and antheraxanthin, in addition to elevated endogenous levels of β-carotene in leaf tissue compared to the other cultivars tested. Results suggest that zeaxanthin, antheraxanthin, and β-carotene may affect drought tolerance in hybrid bermudagrass. Additional research is warranted to help better understand how these antioxidants contribute to the drought tolerance of other turfgrass species and cultivars.

Introduction

Shifts in global climate patterns and population growth in arid and metropolitan regions have resulted in increased water shortages in various regions of the country. As a result, water use restrictions for turfgrass irrigation have increased over the past several years. In some municipalities, turfgrasses are being banned from utilization in residential and commercial landscapes due to their need for supplemental irrigation (SNWA, 2014). In these cases, the environmental benefits that a turfed area can contribute, such as reduce runoff and soil erosion, and remediation of contaminated soils are set aside, and their functional and ecological roles within the landscape (Stier et al., 2013) are lost. In order for turfgrasses to retain their place as part of the modern landscape, the search for characteristics that lead to increased drought tolerance should continue to be an area of intense research.

Hybrid bermudagrasses [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burt-Davy] are widespread throughout the tropical and subtropical regions of the United States (Christians, 1998). Improved hybrid bermudagrasses are known for their improved quality under cold temperatures and tolerance to biotic and abiotic stresses (Taliaferro et al., 2004). Recent breeding efforts have led to the release of cultivars like 'TifTuf' that have demonstrated improved drought and shade stress tolerances (Schwartz, 2014). TifTuf has been identified as having a greater root surface area, volume, and mass at deeper soil depths compared to other cultivars resulting in improved drought avoidance ability (unpublished data, Yurisic, 2016); however, investigations into physiological mechanisms such as antioxidant pigments that might also contribute to drought resistance are lacking.

Drought tolerance is defined as a plant's ability to maintain physiological function when water is limited through processes such as osmotic adjustment, increases in cell wall elasticity,

and antioxidant defense activity (McCann and Huang, 2008a). Drought stress occurs when water availability to the roots is reduced while loss of water to the atmosphere via transpiration continues (Jaleel et al., 2009). Antioxidant systems in plants play integral roles in limiting cell damage caused stress induced reactive oxygen species (ROS). Stomatal closure during drought stress leads to the inhibition of CO_2 diffusion to the carboxylation sites within the leaves where it is assimilated by the RuBisCo enzyme (Inze and Van Montagu, 2002). As a result, NADPH is not consumed in the Calvin cycle and its ability to be used as an electron acceptor during photosynthesis is diminished. When this occurs, the electron transport chain becomes over-reduced as a result of an imbalance between light capture and its use in photosynthesis; especially, when combined with other stresses like high light (Hernández et al., 2012). This imbalance leads to the down-regulation of photosystem II (PSII) activity and results in changes in photochemical efficiency. These changes inevitably lead to excessive amounts of energy present within the antenna complexes of PSII (Reddy et al., 2004). In aerobic conditions, this energy can be transferred from excited state chlorophyll molecules directly to oxygen (O_2), generating the superoxide free radical, O_2^-. The O_2^- radical can be further reduced to form hydrogen peroxide (H_2O_2), and to the most toxic hydroxyl radical, $HO^\bullet$ (Smith et al., 2010). Hydroxyl radicals have a high affinity for all biological molecules; and once formed, are too reactive to be controlled by any other molecules, causing massive cellular damage (Smith et al., 2010). Additionally, intersystem crossing of excited state chlorophyll leads to the formation of triplet chlorophyll which can transfer energy directly to O_2 to produce a very reactive ROS, singlet oxygen ($^1O_2$) (Hernández et al., 2012).

Photosystem I (PSI) and PSII reaction centers in the chloroplast thylakoids are the major generation sites of ROS (Asada, 2006). Reactive oxygen species can react with proteins, lipids,

and DNA causing oxidative damage which disrupts normal cells function. Oxidative stress occurs when the scavenging of ROS by antioxidant systems falls below the production of ROS in situations such as drought, high light, and high or low temperature (Fu and Huang, 2001). The production of ROS is generally linear with the severity of drought stress (Farooq et al., 2009). In order to prevent death, all photosynthetic eukaryotes have evolved effective enzymatic and non-enzymatic antioxidant ROS scavenging systems (Latowski et al., 2011). The utilization of these antioxidants and ROS scavengers enhance plant tolerance to stresses like drought (Vinocur and Altman, 2005).

Enzymatic and non-enzymatic antioxidant systems are main defense mechanisms in plants against the destructive nature of ROS, and when a plant is under stress such as drought, both systems are important (Farooq et al., 2009). The enzymatic defense system consists of the enzymes, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and glutathione peroxidase (GPX). Enzymatic antioxidants are typically compartmentalized within the cell organelles where each enzyme detoxifies a particular type of ROS (Ahmad et al., 2010). The SOD enzyme acts as the first line of defense by converting the O_2^- superoxide radical to H_2O_2. Subsequently, the APX, GPX, and CAT enzymes further act to detoxify the H_2O_2 into H_2O (Apel and Hirt, 2004; Smith et al., 2010).

Non-enzymatic antioxidants which are also capable of detoxifying ROS are secondary plant metabolites including ascorbic acid, glutathione, α-tocopherol, and carotenoids (Ahmad et al., 2010; Ahmad et al., 2009; Apel and Hirt, 2004; Gill and Tuteja, 2010; Huang et al., 2014). Carotenoids are C_{40} tetraterpene molecules synthesized in plastids, and are components of the light harvesting complexes of PSI and PSII (Villar Salvador, 2013). They are lipid-soluble yellow, orange, and red pigments found in plants, algae, fungi, and bacteria (Kopsell and

Kopsell, 2008). Carotenoids can be divided into the hydrocarbon carotenes such as β-carotene (Bc), and the oxygenated xanthophylls such as lutein (Lu), zeaxanthin (Zx), antheraxanthin (Ax), and violaxanthin (Vx) (Jaleel et al., 2009; Kopsell and Kopsell, 2008; Zaripheh and Erdman, 2002). Biosynthesis of carotenoids begins with the dimerization of two C_{20} geranylgeranyl pyrophosphate (GGPP) molecules to form phytoene (Cunningham Jr and Gantt, 1998). Desaturation of phytoene and subsequent cyclization steps leads into branching in the pathway with the formation of either α-carotene (which later converts into lutein), or β-carotene (which later converts into Zx, Ax, and Vx) (Kopsell et al., 2009; McElroy and Kopsell, 2009) (Figure 8).

All photosynthetic pigments are important for light harvesting; however, carotenoids have additional roles that may help plants tolerate drought such as protection from oxidative damage (Jaleel et al., 2009). The accessory pigment, β-carotene is an antioxidant that can effectively protect photochemical machinery through direct quenching of triplet state chlorophyll (Farooq et al., 2009). Lutein and other carotenoids are responsible for the quenching of excited state chlorophyll by harvesting energy and releasing it as heat through thermal relaxation, which prevents the formation of O_2^- (Jahns and Holzwarth, 2012). The dissipation of energy from excited state chlorophyll in the form of heat is termed non-photochemical quenching (NPQ), and strong correlations exist between the carotenoids Zx and Ax and NPQ (Adams et al., 1995; Latowski et al., 2011). Carotenoids can directly quench other ROS such as O_2^- and H_2O_2 (Hernández et al., 2012). The antioxidant property of carotenoids is primarily a result of their conjugated double-bond structure which enables them to interact with ROS to delocalize their reactive unpaired electrons (Ahmad et al., 2010).

Three carotenoid pigments, Zx, Ax, and Vx, comprise the xanthophyll cycle. In the xanthophyll cycle, Vx is de-epoxidized to Zx through the intermediary, Ax. These reactions are

catalyzed by the violaxanthin de-epoxidase enzyme (VDE) in high light conditions. In low light, the reverse occurs with the epoxidation of Zx to Ax to Vx as facilitated by the zeaxanthin epoxidase (ZE) enzyme (Demmig-Adams et al., 1996). Violaxanthin de-epoxidase activity is strongly influenced by increases in the inter-lumen pH of the thylakoid membranes of chloroplasts during periods of stress when the electron transport system is saturated (Jahns and Holzwarth, 2012). The amount of Zx is dependent upon the amount of activity of VDE compared to ZE, with increased accumulation of Zx under intense light (Müller et al., 2001). Since the total amount of Ax and Zx is highly related to NPQ, researchers often report the ratio of Ax plus Zx to total xanthophyll cycle pigments using the formula: (Zx+Ax)/(Vx+Ax+Zx), the de-epoxidation state (DEPS) of the xanthophyll cycle. Plants are able to reduce excessive energy in PSII through NPQ by increasing the concentration of xanthophyll pigments (especially Zx and Ax) to serve as a protective mechanism for plants under stress conditions (Reddy et al., 2004).

Studies examining the activity of carotenoids during drought have been conducted on several plant species. Concentration of carotenoids per unit of chlorophyll increased under periods of drought stress which resulted in greater photo-protection in rosemary (*Rosmarinus officinalis*) (Munné-Bosch and Alegre, 2000a). In *Arabadopsis*, rice (*Oryza sativa*), and tobacco (*Nicotiana tabacum*), overexpression of genes that elevate the biosynthesis of Bc resulted in enhanced resistance to drought stress due to increases in NPQ compared to the wild types (Davison et al., 2002; Du et al., 2010; Wu et al., 2015). In severely drought stressed strawberry trees (*Arbutus unedo* L.), Zx increased by 75% while chlorophyll decreased by a similar proportion which resulted in reduced light harvesting capacity and increased protection against oxidative stress (Munné-Bosch and Peñuelas, 2004). In Chinese dwarf cherry (*Cerasus humilis*) seedlings, greater accumulation of Zx and Ax compared to well-watered controls provided

photoprotection during prolonged water stress, which promoted enhanced survivability of stressed plants (Song et al., 2011). Similarly, in the drought tolerant species of photosynthetic algae (*Ulva pertusa*), greater accumulation of Zx and Ax were observed in desiccated thalli compared to hydrated thalli resulting in increased NPQ and DEPS (Xie et al., 2013).

In turfgrasses, the majority of drought research pertaining to antioxidant systems has focused mainly on the enzymatic antioxidants (DaCosta and Huang, 2007; Du et al., 2009; Fu and Huang, 2001). Findings from these studies suggest that improved drought and heat tolerance may be related to increased activity of antioxidant enzymes. Enzymatic antioxidant activity is easily quantified using spectrophotometric methods. However, measurements of individual carotenoid pigments are more difficult and time-consuming to obtain requiring the use of liquid chromatography with mass spectrometry, which is one potential reason why carotenoids have received less attention (McElroy and Kopsell, 2009).

Carotenoids accumulate during periods of drought in a variety of plant species; however, limited information is available regarding the role of carotenoids turfgrasses under stress. McElroy et al. (2006) determined that xanthophyll cycle pigments increased in creeping bentgrass (*Agrostis stolonifera* L.) under high irradiance, and that selection of grasses with higher quantities of carotenoids may help turfgrass breeders identify more stress-tolerant species. Other studies have related carotenoid pigments to herbicide injury in turfgrasses (Elmore et al., 2011; Mayonado et al., 1989; McCurdy et al., 2008) and corn (*Zea mays* var. *rugosa*) (Kopsell et al., 2011). Due to the photo-protective role of carotenoids, identifying turfgrasses with increased carotenoid concentrations during drought stress may help turfgrass breeders select for more drought tolerant genotypes.

One tool that many researchers have adopted for use in turfgrass drought research is digital image analysis (DIA). Digital image analysis provides a rapid method to objectively quantify parameters such as turfgrass color (Karcher and Richardson, 2003) and percent green cover (Richardson et al., 2001). In summary, DIA consists of three steps: (i) acquiring a quality image with consistent light source and camera settings; (ii) selecting the desired area of the image to quantify; and (iii) quantifying the parameters of interest from the image selection area (Karcher and Richardson, 2013). Specific computer software is then used to analyze each image based on an individual pixel basis. Utilizing a specific image analysis algorithm, software such as SigmaScan (Systat Software, Chicago, IL) assigns red, blue, and green (RBG) values to individual pixels based upon specific color threshold limits set within the program (Karcher and Richardson, 2005). Since RGB values are not easily relatable to how the human eye perceives color, they are converted to the more intuitive hue angle, saturation, and brightness (HSB) color scale. Hue is a measure of all colors visible to the human eye based on a continuous circular 0 - 360^0 scale, saturation is the purity of the color, and brightness is the relative lightness or darkness of the color (from black to white). During image analysis, green pixels (typically hues between 60^0 and 120^0 for turfgrasses) are distinguished from other pixels outside of the hue range by the software (Karcher and Richardson, 2013). Turfgrass cover can then be calculated as a percent based on the ratio of green pixels selected to the total number of pixels in the entire image (Richardson et al., 2001). The HSB parameters can be further converted into a measure of how dark green the color is on a scale of 0 to 1which is known as the dark green color index (DGCI), with values closer to 1 representing darker green color (Karcher and Richardson, 2003).

The ease of acquiring DIA information has made its utilization in research far reaching, and it has become one method of measuring stress in turfgrass. It is often used to quantify the

loss of green color in plant tissue during drought conditions (Karcher et al., 2008; Richardson et al., 2008; Steinke et al., 2009; Zhou et al., 2013). Dark green color indices have also been used in several studies to relate green color to various inputs and environmental factors. Karcher and Richardson (2003) determined that DGCI was affected by nitrogen source and rates in zoysiagrass (*Zoysia japonica* Steud.) fairways in addition to nitrogen rates in creeping bentgrass putting greens. Dai et al. (2009) reported that DGCI values were reduced in salt-stressed annual bluegrass (*Poa annua* L.) putting greens. Githinji et al. (2009) used DIA to determine that hybrid bluegrasses (*Poa pratensis* L. x *P. arachnifera* Torr.) yielded higher DGCI values compared to tall fescue (*Festuca arundinacea* Shreb.). Additionally, DGCI was found to be negatively correlated to wear tolerance, whereas percent green turfgrass cover was directly correlated to wear tolerance for four hybrid bermudagrasses cultivars under traffic (Kowalewski et al., 2015).

Similar methods using tristimulus colorimetry [L*a*b*] devices in vegetable crops have been able to establish correlations between color values and carotenoid pigment concentrations (Ameny and Wilson, 1997; Itle and Kabelka, 2009; Seroczyńska et al., 2006) with varying results. According to Seroczyńska et al. (2006), flower color of winter squash (*Cucurbita maxima* Duch.) was strongly correlated to carotenoid content, but weakly correlated in the fruit flesh. Itle and Kabelka (2009) reported strong correlations existed between a* and b* color values to carotenoids which could provide a fast and affordable method to estimate carotenoid content in pumpkins and squash (*Cucurbita* spp.). However, relationships between color and pigment concentrations in turfgrasses have yet to be reported.

Carotenoids are effective scavengers of ROS, and increases in the induction of their synthesis may enhance drought tolerance (Davison et al., 2002; Jaleel et al., 2009; Munné-Bosch

and Alegre, 2000b; Wu et al., 2015). Researchers may be able to improve stress tolerance in turfgrasses by selecting or engineering plants that have increased carotenoid content in their tissues (McElroy and Kopsell, 2009). Using DIA color analysis, researchers may be able to more quickly estimate pigment content in leaf tissue compared to high-performance liquid chromatography (HPLC). New drought tolerant cultivars may be able to withstand drought stress due to higher accumulations of carotenoid pigments within leaf tissue. Therefore, the objectives of this research were to: (i) compare changes in pigment concentration among three commercially available cultivars of hybrid bermudagrass under drought stress; (ii) determine if differences in carotenoid concentrations among cultivars influence drought tolerance; and (iii) determine if a relationship was present among pigment concentrations and DIA color values.

Materials and Methods

Plant culture

The three hybrid bermudagrass cultivars chosen for this investigation were TifTuf, Latitude 36, and Tifway. Tifway is an older cultivar released in the 1950's (Burton, 1966) and is often used as a standard check in bermudagrass research. On 15 September 2014, round plugs (5 cm in diameter × 5 cm deep) were obtained from field plots established on Sequatchie silt loam soil (fine-loamy, siliceous, Typic Paleudults) at the East Tennessee Research and Education Center (ETREC) using a manually-operated sod plugger. Plugs were transferred into plastic trays (5 cm in depth) filled with a peat-based potting mix (Pro-Mix Bx, Fafard, Inc.). 30 plugs of each cultivar were planted into two separate trays. Trays were placed in a glasshouse at the University of Tennessee Institute of Agriculture (UTIA) to allow the plugs to increase above and belowground biomass. Glasshouse conditions measured 35° / 21° C day/night air temperature

with an 18 h photoperiod. Individual trays were supplied nutrients once every 2 weeks via a 300 ml solution of water soluble fertilizer (UltraSol, 20 N – 20 P_2O_5 – 20 K_2O, SQM Co., Atlanta, GA) at a rate of 25 kg N ha^{-1}. All plants were hand-trimmed to a height of 10-cm using hand shears and clippings were returned. Supplemental water via an overhead irrigation system was set to run four cycles per d for 15 min each cycle to maximize growth and vigor.

Lysimeters were constructed with 10.3-cm (inside diameter) PVC sewer pipe cut into 45-cm sections. Landscape fabric was cut into approximately 15 × 15 cm squares and placed over one end of each pipe section. The edges of the fabric were pulled firmly over the end of the pipe to cover the entire opening. Outdoor-grade duct tape was used to secure the fabric to the outside of the pipe, creating a water-permeable layer that allowed for water drainage, yet was sturdy enough to hold the growing media inside (Figure 1). Lysimeters were filled with dry 100% silica sand (Short Mountain Silica, Mooresburgh, TN) which was graded to the particle size specifications of the United States Golf Association (Hummel, 1993). All lysimeters were filled with 6.18 kg of dry sand in order to obtain a relatively uniform root zone bulk density of 1.57 g cm^{-3} (Figure 2).

Vegetative propagation of plants began on 26 May 2015 under the same growing conditions as previously discussed, except irrigation was increased to eight 15 min cycles per d. Plants consisting of three tillers each were removed from the trays and placed in a water bath to remove any soil/potting mix left adhering to the roots. Plants were uniformly trimmed using scissors to a height of 8-cm, and roots were trimmed to a length of 1-cm before being planted. Eight evenly spaced plants were inserted into approximately 7-cm deep pre-bored holes into the sand of each lysimeter. Lysimeters were randomly assigned position within the greenhouse bench area and were shifted twice per week to help eliminate possible micro-environmental

effects (Figure 3). Plants were provided with a controlled release granular fertilizer (Greenlinks 24 N – 4 P_2O_5 – 12 K_2O, Tyler Enterprises, Morris, IL) 1 week after propagation at a rate of 50 kg N ha^{-1}.

After 5 weeks, lysimeters were transported to ETREC to allow plants to complete establishment in the field environment conditions which this experiment was to be conducted. Plants were considered to be completely established once all lysimeters had approximately 100% turfgrass canopy coverage based on visual assessment. Lysimeters were supplied water from an in-ground system set to deliver 15 min of water at 2 h intervals 8 times per d. All lysimeters were fertilized again with the same controlled release granular fertilizer used previously at a rate of 50 kg ha^{-1}. Due to the low nutrient holding capacity of the sand media (3.24 meq 100 g^{-1} of soil), a diluted rate (5 kg N ha^{-1}) of the same 20 N – 20 P_2O_5 – 20 K_2O fertilizer solution used previously was applied five times per week as a foliar spray at an application volume of 2.5 ml per lysimeter using a hand spray bottle. This was to help offset the loss of soil applied nutrients due to leaching through the sand matrix. Plants were trimmed using scissors two to three times per week at a height of 3.8-cm, and clippings were returned. Lysimeters were kept in the same random configuration as in the glasshouse, and were shifted on days when plants were trimmed to reduce any potential effects of micro-environment.

Rainout box

Two small rainout boxes (1.2 m L × 0.9 m W ×0.5 m H) were constructed to the same height as the lysimeters using 1.3-cm thick plywood to serve as the walls. A lid with clear acrylic roofing panels attached to a hinged frame was fastened to the box at the top of one of the walls (Figure 4). The purpose of the lid was to protect the plants inside from rain, and was

designed to be easily opened and closed manually as needed. The clear top allowed light to enter during periods when the lid was closed. Latches securing the lids to the rainout boxes helped prevent the lids from blowing open in the event of elevated wind conditions. To insulate the sides of the box from excessive heating due to direct sun radiation exposure, a double reflective radiant barrier (Reflectix, Markleville, IN) was stapled to the inside and outside faces of the walls of each box (Figure 4).

Drought Conditions

Drought stress was imposed by withholding all rain and irrigation from the lysimeters for a period of 28 days beginning on 3 Aug 2015 at ETREC and 5 Aug 2015 at UTIA. The rainout box lid remained open during the study between 0800 and 1700 h every day to expose plants to natural sunlight, temperature, humidity, and wind conditions. To ensure no water was supplied to the plants during the drought period, the rainout box lid was closed over the lysimeters during periods of rain, as well as at night when the researchers were not present. A weather data logger (WatchDog 1450 series, Spectrum Technologies, Aurora, IL) was attached to each rainout box to record the average daytime air temperature and humidity, in addition to photosynthetically active radiation (PAR) in 1 h increments each day between 0800 and 2000 h.

Experimental Design

Ten weeks following propagation, all lysimeters had reached approximately 100% visual turfgrass canopy coverage. Measurements on all dependent variables were obtained at 4 d intervals after withholding water (DAWW) with initial sampling at 0 DAWW. Sampling at 0 DAWW was carried out to provide base cultivar reference for each dependent variable. The experiment was set up as two simultaneous runs with each rainout box being separated by

location. One location was at ETREC in an area of full sun and wind exposure among plots at the University of Tennessee's Center for Athletic Field Safety (35°54'05.2" N Lat., 83°57'34.5" W Long.). The second location was on the UTIA campus in a site surrounded by nursery plant greenhouses (35° 56' 46.3" N Lat., 83° 56' 18.6" W Long.), which provided greater protection from wind in addition to shaded conditions in the early morning/late afternoon. In order to allow for adequate time to obtain data at each location, the trial start dates were offset by 2 d with sampling at ETREC beginning on 3 Aug 2015, and on 5 Aug 2015 at UTIA. Sampling of plants occurred at approximately 1200 h on each date. Trimming of plant foliage with scissors ceased after trial initiation in order to eliminate potential water loss through leaf wounding.

A total of 72 lysimeters were arranged in a completely randomized experimental design using three replications for each sampling date within each rainout box. Destructive sampling of leaf tissue was required throughout this study in order to obtain measurements of physiological parameters. Therefore, nine lysimeters (three replications of each variety) were removed from the rainout box for evaluations on each of eight total sampling dates. Once sampling was complete, the lysimeters were placed back into position within their respective rainout box; however they were not used for any subsequent research. Lysimeters were pre-randomized in regards to their position within the rainout boxes as well as the order of their sampling date using Microsoft Excel (Excel 2010, Microsoft Corp., Redmond, WA). A split-split plot treatment design was applied to the experiment with location serving as the whole plot factor, sampling date as the sub-plot factor, and cultivar as the sub-sub-plot factor.

At the initiation (0 DAWW) of the study at each location, lysimeters were watered to field capacity at approximately 1100 h to ensure all plants began drought treatment with similar soil moisture content. First, lysimeters were completely submerged in a water bath to a level

slightly above the top edge of the lysimeter. Air bubbles observed rising to the surface from the lysimeter indicated that water was infiltrating the pore spaces, forcing all air upward and out of the sand column. After a period of approximately 15 min, air bubbles were no longer visibly rising from the surface of the lysimeters, at which point the rootzone was considered to be at complete saturation. Lysimeters were then placed on a porous gravel surface to allow the water to drain freely. After a period of approximately 30 min, water had ceased dripping from the bottom of the lysimeter and were considered to be at field capacity.

Data Collection

Data were collected on nine total lysimeters (three per cultivar) selected within each sampling date. Turfgrass quality was assessed visually in the field according to Morris and Shearman (1998) using a 1 to 9 scale (1 = lowest possible quality, 9 = ideal quality, 6 = minimum acceptable turfgrass quality). After assessing TQ, plants were taken into the lab on the UTIA campus for further evaluations. Percent green turfgrass cover (GC) and dark green color index (DGCI) measurements were obtained using digital image analysis (DIA) similar to Richardson et al. (2001) and Karcher and Richardson (2003). Lysimeters were placed inside a box illuminated with two 9-watt 6500 °K compact fluorescent lightbulbs (TCP 48909, Technical Consumer Products Inc., Aurora, OH) which provided the constant ambient lighting conditions necessary for DIA image acquisition (Figure 9). A digital camera (CoolPix P7100, Nikon Corp., Tokyo, Japan) was used to acquire images through an aperture cut in the top of the box directly above each lysimeter at 3648 × 2736 pixel resolution with an f/4 F-stop, 1/8 sec. exposure time, and ISO-100 shutter speed setting.

Fluorescence parameters were obtained using a pulse-amplitude-modulated (PAM) fluorimeter (Open-FluorCam FC 800, Photon Systems Instruments, Brno, Czech Republic). Whole plants were dark acclimated for approximately 1 h before analysis to fully oxidize the reaction centers of PSII. Measurements of minimal fluorescence (F_0) were taken with all reaction centers in the fully "open" state of dark acclimation. An intense flash (3000 µmol m^{-2} s^{-1} for 0.1 µs) of light leads to the over-reduction of the electron transport chain, which transiently "closes" all reaction centers. All photochemical quenching is thereby eliminated, resulting in the maximum fluorescence yield (F_M). Variable fluorescence (F_V) was determined as: $F_V = F_0 - F_M$, and the maximum quantum yield of PSII was then calculated as the ratio of F_V to F_M (F_V/F_M) (Kopsell et al., 2011; PSI, 2006).

Relative water content (RWC) of leaf tissue was measured according to the method of Barrs and Weatherley (1962). Approximately 0.1 g of fresh leaf tissue was excised from each lysimeter and weighed immediately to obtain fresh weight (FW). The leaves were then soaked in deionized water in a closed petri dish for at least 24 h at 4 °C. After soaking, leaves were blotted dry with a paper towel and weighed to obtain the turgid weight (TW). Leaves were then dried in an oven at 72 °C for 48 h prior to being weighed again to determine dry weight (DW). The formula used to calculate RWC was: (FW/DW) / (TW-DW) × 100.

To quantify tissue pigments, leaf tissue was harvested from lysimeters using hand shears at a canopy height of approximately 1.0-cm. All leaf tissue was immediately stored at -80 °C to await pigment extraction and analysis. Leaf tissue pigments from a 0.25 g fresh weight sample were extracted and quantified similar to previously published methods (Brosnan et al., 2011; Elmore et al., 2011; Kopsell et al., 2007). An Agilent 1200 series high performance liquid chromatography unit with a photodiode array detector (Agilent Technologies, Palo Alto, CA)

was used for pigment separation and quantification. A 0.8 ml aliquot of ethyl-β-apo-8´-carotenoate internal standard (Sigma Chemical Co., St. Louis, MO) was added to each sample to determine extraction efficiency. A 250 × 4.6 mm RP-C_{30} column with a 10 × 4.0 mm guard cartridge (ProntoSIL; MAC-MOD Analytical Inc., Chadds Ford, PA) was used for chemical separation of similar carotenoid compounds. Separations were achieved using a binary mobile phase of 11% methyl *tert*-butyl ether, 88.99% MeOH, and 0.01% trimethylamine (v/v/v) at a flow rate of 1.0 ml·min^{-1}. Carotenoids, chlorophyll *b*, and the internal standard were detected at 453 nm; and at 652 nm for chlorophyll *a*. All pigment data presented hereafter are expressed as µg·g^{-1} fresh weight of the leaf tissue.

Statistical analysis

All data were subjected to analysis of variance using the GLM procedure in SAS version 9.4 (SAS Institute, Cary, NC) to test for treatment main effects and interactions. When appropriate, means were separated using Fisher's protected LSD at the $P \leq 0.05$ level of significance. Scatter plots of GC and TQ data over time resulting in nonlinear relationships. Data points were fitted using a nonlinear variable-slope sigmoidal model according to Karcher et al. (2008). The model was used to predict the number of days required to produce a drought response of any desired magnitude of interest of the maximum possible value of GC (GC_X) or TQ (TQ_X), where: $Y=(100)/((1+10^{(DaysX-DAWW)}) \times Slope))$. In this equation, Y was equal to GC, 100 indicated the maximum level of GC or TQ, $Days_X$ was the number of estimated days to produce a response of the maximum potential GC or TQ, and the slope variable showed the rate of change in GC or TQ. A sum of squares F-test was applied to the model to compare the differences in model parameter estimates between global (shared $Days_X$) verses individual

(Days$_X$ for each cultivar), and if the test was significant ($P \leq 0.05$), then cultivar effects were determined to be significant (Steinke et al., 2011). Parameter estimates were then used to calculate 95% confident intervals for the number of DAWW until each cultivar reached 50, 55, and 75% green cover, or TQ = 6 in response to drought. Entries were considered significantly different if their confidence intervals did not overlap at each green coverage or TQ percent value (Karcher et al., 2008). Regression analyses were performed using GraphPad Prism version 6.05 (GraphPad, San Diego, CA) and treatment means were plotted across sampling dates with standard error bars presented as a means of statistical comparison. Pearson correlations between pigments and color values were carried out using the CORR procedure in SAS version 9.4. No significant location-by-treatment interactions were detected in the fluorimeter data, in addition to TQ, GC, RWC, Bc, total chlorophyll (TCh), Total carotenoids (TCa), and the ratio of total carotenoids to total chlorophyll (TCa/TCh); therefore, means presented hereafter for these variables are pooled across both locations.

Results and Discussion

Main effect and/or interactions involving location were significant ($P \leq 0.05$) for several parameters involving the xanthophyll pigments; therefore, these data were separated by location. It is important to note that on several occasions, measurements of PAR were much higher on samplings dates at UTIA compared to at ETREC (Figure 10). This was expected since data collection at each location was offset by 2 d, resulting in different light conditions among sampling dates between locations. It is well documented that carotenoids (especially the xanthophylls) are highly sensitive to changes in light intensity (Demmig-Adams, 1990; Demmig-Adams et al., 1996; McElroy et al., 2006; Müller et al., 2001). Within the same sampling dates,

changes in PAR were similar to fluxes in xanthophyll pigments at both locations. Recorded temperature and humidity data at each location are displayed in Figure 5.

Drought resistance characteristics among the three cultivars used in this study differed markedly over the 28 d drought period. Significant ($P \leq 0.05$) day-by-variety interactions existed for TQ, GC, and RWC. Nonlinear regression revealed that model parameters differed ($P \leq 0.001$) among cultivars for TQ and GC indicating cultivar effects were significant. The GC_{50} values of TifTuf did not differ from Latitude 36 or Tifway; however significant differences were detected among cultivars for GC_{55}, and GC_{75} (Table 3). The amount of time for TifTuf to reach the minimum acceptable TQ_6 rating (TQ = 6) was 17 DAWW, which was significantly ($P \leq 0.05$) longer compared to 9.1 and 6.1 DAWW for Latitude 36 and Tifway (Table 4). These responses illustrate the greater drought tolerance of TifTuf compared to Latitude 36 and Tifway. Relative water content for Latitude 36 was significantly less than TifTuf and Tifway on 8 and 16 DAWW. RWC was the same for all varieties on all other dates, except at 28 DAWW when TifTif had the lowest RWC (Figure 12). No significant differences were detected for DGCI among cultivars; however, there was a significant decline in mean DGCI for all cultivars from 0 to 28 DAWW (Figure 13). Higher ($P \leq 0.05$) TQ_6, $GC_{55, 75}$ values, as well as overall TQ and GC across 28 days of drought demonstrate the greater drought tolerance of TifTuf compared to Latitude 36 and Tifway.

Measurements of F_0 and F_M were similar among all cultivars with significant initial declines in F_0 and F_M between 0 and 4 DAWW. Overall, a 55% decrease in F_0 occurred between 0 to 28 DAWW, accompanied by a 72% decrease in the value of F_M (Figure 14). Such changes resulted in a significant day-by-variety interaction in the F_V/F_M data. Average F_V/F_M remained similar for all cultivars from 0 to 20 DAWW; however, F_V/F_M of TifTuf dropped sharply from

0.653 to 0.300 between 20 and 28 DAWW corresponding to a significant drop in RWC from 78 to 27% (Figure 14). Similar studies (Munné-Bosch and Alegre, 2000a; Munné-Bosch and Cela, 2006) reported that F_V/F_M was significantly reduced only in severely stressed plants having very low RWC percentages. There was an approximate 16% decline in F_V/F_M values for Latitude 36 and Tifway between 0 and 28 DAWW, respectively (Figure 14); however, RWC values for these two cultivars did not drop below 42% at any point in time (Figure 12). The cultivar, TifTuf has been reported to consume more water volume compared to Latitude 36 and Tifway in concomitant research (unpublished data, Yurisic, 2016). Therefore, the large drop in RWC for TifTuf could be a result of the plant exhausting all available water within the lysimeter before the other two cultivars. Photosynthetic rate is known to decrease as RWC decreases as a result of stomatal and metabolic limitations (Jaleel et al., 2009). In hybrid bermudagrass, improved photosynthetic capacity could be attributed to early stomatal closure coupled with the maintenance of higher RuBisCo enzyme activity and synthesis at later stages of drought (Hu et al., 2009). Thus, sustained photochemical efficiency of Latitude 36 and Tifway may have been manifested as a result of early stomatal closure resulting in the maintenance of higher RWC in the leaves in addition to increased carbon metabolism activity; however, further investigation is needed for confirmation.

Total chlorophyll (measured as total combined chlrophyll *a* and chlorophyll *b*) did not differ among cultivars on any sampling date over the 28 d drought period. Average TCh increased slightly from 2624 to 3111 μg g^{-1} between 0 and 8 DAWW. However, from 8 to 28 DAWW, average TCh decreased for all cultivars by 63% (Figure 15). Turfgrass chlorophyll loss is a common indication of drought stress, and is a likely result of photo-oxidation of the chlorophyll pigment molecules (Fu and Huang, 2001; Hatamzadeh et al., 2014). Furthermore,

chlorophyll degradation is regarded as a protective measure utilized by stressed plants to reduce light harvesting capacity which would allow for increased NPQ energy dissipation (Munné-Bosch and Alegre, 2000a; Munné-Bosch and Alegre, 2000b).

Zeaxanthin, Ax, Vx, and Lu are xanthophyll pigments that play key roles in the dissipation of excess light energy in PSII during stress (Demmig-Adams, 1990; Jahns and Holzwarth, 2012). A day-by-variety interaction existed for Zx, DEPS, and the ratio of Zx + Ax to chlorophyll (ZA/Chl); however, these interactions varied between locations. Between 0 and 24 DAWW, Zx concentrations among all cultivars increased from 3.30 to 37.75 μg g^{-1} at ETREC; whereas at UTIA, the increase was from 4.39 to 35.17 μg g^{-1}. Maximum Zx concentrations were reached at approximately 24 DAWW at both locations, followed by a sharp drop at 28 DAWW (Figures 16 and 17) Concentrations of Zx were higher for TifTuf and Latitude 36 compared to Tifway at ETREC (Figure 16), and was highest for TifTuf at UTIA (Figure 17) on the 24 DAWW sampling date. Due to the role Zx plays in photoprotection, the observed increases of Zx may be responsible for the enhanced GC and TQ of TifTuf at more severe stages of drought. Similar results were observed by Munné-Bosch and Peñuelas (2004) where large increases in Zx at severe stages of water stress resulted in greater protection against drought-induced oxidative stress.

No differences among cultivars were detected for Violaxanthin or for the combined amounts of Vx, Ax, and Zx (VAZ); however concentrations decreased significantly between 0 and 28 DAWW. At both locations, Vx declined significantly between 0 and 28 DAWW (Figure 18). A 55 and 60% decrease in Vx resulted in a 47 and 44% drop in total VAZ at ETREC and UTIA, respectively (Figure 19). Because Vx was present in much higher concentrations within

the leaf tissue under well-watered conditions, the severe drop in Vx most likely produced the significant reduction of the total VAZ in the xanthophyll cycle pigment pool.

Violaxanthin is converted to Ax and Zx in the xanthophyll cycle (Demmig-Adams and Adams III, 1996b). In stressed plants, the activation of DEPS results in enhanced photoprotection through a direct transfer of light energy from the light harvesting complexes of PSII to Zx and Ax (Latowski et al., 2011; Morales et al., 2008). In our study, increased activity of the xanthophyll cycle led to concurrent decreases in Vx alongside increases in Zx in addition to the ratio of ZA/VAZ in all cultivars. However, TifTuf exhibited higher DEPS at 28 DAWW at ETREC (Figure 20) and at 24 and 28 DAWW at UTIA (Figure 21). Observed changes in ZA/TCh appeared to be similar to the changes in DEPS over the 28 d drought period. The ZA/TCh ratio increased with maximum levels reached with the most severe drought stress at 24 DAWW at ETREC (Figure 22), and at 28 DAWW at UTIA (Figure 23). A study conducted in spinach (*Spinacia oleracea* L.) by Verhoeven et al. (1997) suggested that decreases in chlorophyll accompanied by increases in ZA allowed for higher energy dissipation per unit of light intercepted, which led to enhanced stress tolerance. In this study, DEPS and ZA/TCh had a significantly strong positive correlation ($r = 0.96$, $P \leq 0.001$, n = 144) with increased DEPS activity leading to a higher ZA/TCh ratio. Reductions in TCh along with increased DEPS of TifTuf resulted in more Zx and Ax per unit of chlorophyll compared to the other cultivars. Non-photochemical quenching is known to be highly related to DEPS, promoting greater photoprotection during stress (Demmig-Adams, 1990; Demmig-Adams et al., 1996; Song et al., 2011). Additional photoprotective effects are likely due to the scavenging of ROS by amplified concentrations of Zx and Ax (Müller et al., 2001). Evidence presented in this study suggests that

improved drought tolerance of TifTuf could be related to the increased concentration of Zx and Ax, thereby increasing its capacity of NPQ-dependent photoprotection.

β-carotene (Bc) is an accessory pigment that plays a direct role in photoprotection through quenching of triplet state chlorophyll, thereby preventing the generation of ROS (Jaleel et al., 2009). Because Bc is also a precursor to the xanthophyll cycle pigments, Zx, Ax, and Vx, overexpression of the Bc hydroxylase gene (*chyb*) can dramatically enlarge the xanthophyll cycle pool and improve drought resistance (Davison et al., 2002; Wu et al., 2015). In the present study, there was a significant ($P = 0.015$) day-by-cultivar interaction for Bc concentration (Figure 24). Average Bc levels for all cultivars combined decreased 64% between 0 and 28 DAWW. Similar results have been reported in other plant species (Munné-Bosch and Alegre, 2000a; Munné-Bosch and Cela, 2006).

Interestingly, initial Bc levels for TifTuf were approximately 30% less than Latitude 36 and Tifway. There was a decline in Bc concentrations from 4 to 28 DAWW of approximately 70 and 50% for Latitude 36 and Tifway, respectively. However, between 0 and 8 DAWW, the Bc concentration of TifTuf increased dramatically reaching a peak concentration at 16 DAWW (a 39% increase from 0 DAWW). At 20 DAWW, Bc concentration of TifTuf increased to levels 27 and 20% higher than that of Latitude 36 and Tifway, until rapidly declining between 20 and 28 DAWW. According to the findings of Munné-Bosch and Alegre (2000b), the inability of plants to increase levels of antioxidants such as Bc and α-tocopherol can lead to more severe damage at later stages of drought. In our study, Bc levels in the earlier drought stages for TifTuf increased while the levels for Latitude 36 and Tifway decreased (Figure 24). Concentrations of Bc were positively related ($P \leq 0.001$) to GC and TQ ($r = 0.85$ and $r = 0.78$, n = 144) which indicates a possible role of Bc in sustaining hybrid bermudagrass quality during drought. β-

carotene also plays a direct role in the scavenging of the single oxygen ($^1O_2$) ROS (Ahmad et al., 2010; Asada, 2006). Therefore, increased accumulation of endogenous levels of Bc in TifTuf leaves may have been responsible for greater increases in Zx and Ax, allowing for greater ROS scavenging and photoprotection through NPQ.

Due to the photoprotection afforded by carotenoids, it was expected that cultivars with higher carotenoid concentration would be better able to prevent photo-oxidation of chlorophyll, thereby sustaining green color longer during drought. However, assessments of both DGCI and chlorophyll content remained similar for all cultivars while GC values of TifTuf were higher than the other cultivars between 8 and 20 DAWW (see figures 13 and 15). Total carotenoids throughout this study were highly related to TCh ($r = 0.93$, $P \leq 0.001$, n = 144) and GC ($r = 0.79$, $P \leq 0.001$, n = 144). There was a significant ($P = 0.0126$) day-by-cultivar interaction observed for TCa (Figure 25). Changes in TCa over time were similar to that of Bc with initial levels of TCa in TifTuf approximately 36% less than the other cultivars. Total carotenoids declined steadily between 0 and 28 DAWW for Latitude 36 and Tifway; whereas in TifTuf, TCa was significantly elevated by 23% between 0 and 16 DAWW before beginning a decline at the later stages of drought.

It is important to note that GC analyses are based on green color per unit area whereas pigments were extracted from a homogenized sample of the total leaf tissue removed from the plant. A possible explanation in the discrepancy between chlorophyll content and GC could be due to the distribution of pigmentation in the leaves. As plants experience water stress, leaves become increasingly senescent as nutrients are mobilized to younger tissues (Munné-Bosch and Alegre, 2004). It is possible that the amount of completely senesced leaves in cultivars like Latitude 36 and Tifway was higher than in TifTuf at each sampling date. Therefore, chlorophyll

pigments would have been extracted predominantly from a few young, non-senesced leaves in Latitude 36 and Tifway, whereas in TifTuf, chlorophyll would have been extracted from several leaves that had retained at least some chlorophyll pigment. The elevated GC during drought for TifTuf suggests that less leaf senescence may have occurred compared to the other cultivars. Further investigation is needed to confirm if differences in pigment distribution throughout the canopy exists among cultivars during drought stress.

Additionally, because carotenoids are lipid-bound molecules and chlorophylls are water soluble, chlorophyll molecules are more susceptible to drying conditions. It has been established that carotenoids play additional roles in protection through the maintenance of chloroplast membrane stability during stress (Havaux, 1998; Latowski et al., 2011; Müller et al., 2001). In addition to the increased ROS scavenging ability, plants such as TifTuf that can accumulate more carotenoids may also be able to survive drought by sustaining the integrity of cellular membranes until plants are rehydrated. Future antioxidant research should investigate the extent of the relationship between membrane stabilization and carotenoids in turfgrasses during drought.

Although DGCI values did not differ by cultivar throughout the entire study, the decline in DGCI over time indicated that drought induced changes in turfgrass color among cultivars. Combining all cultivars together, there were significant correlations ($P \leq 0.05$) on each sampling date between DGCI and the TCh, Lu, Bc, and TCa pigment variables, with one exception for Lu ($P = 0.10$) at 8 DAWW Table 5). However, relationships among these variables were only weak to moderate ($r \leq 0.67$) until 8 DAWW. From 8 to 20 DAWW, Bc was moderate to strongly correlated to DGCI ($r = 0.68$ to 0.89). This relationship weakened ($r \leq 0.66$) as drought progressed beyond 20 DAWW. From 16 to 28 DAWW, Lu was strongly associated with DGCI

($r \geq 0.72$). Similarly, TCh and TCa were moderate to strongly related to DGCI ($r = 0.68$ to 0.91) from 16 to 28 DAWW. It is unclear why correlations were not as strong in the first stages of drought. One hypothesis is that higher water content within the leaves may have affected the way light scattered within the leaf tissue compared to drier leaves. This could perhaps have created a washing out effect in color as perceived by the digital camera, compared to the undiluted pigmentation of dry leaf tissue. Results of these analyses suggest that the estimations of concentrations of TCh, Lu, Bc, and TCa may only be reliable at later stages of drought.

It is important to note that the most drought tolerant cultivar, TifTuf, did not possess as high of a concentration of Bc, Zx, or TCa at the earlier stages of drought compared to the less drought tolerant cultivars. However, TifTuf did accumulate higher levels of these pigments as drought stress increased over time. This suggests that estimations of carotenoid content at the early drought phases may not be sufficient to predict drought tolerance among cultivars of the same species. Therefore, if estimations of pigment concentration are to be made; they may be more accurate at the later stages of drought since changes in pigments among cultivars were significant across time.

Interestingly, when correlations between pigments and the three DIA color parameters, hue, saturation, and brightness were analyzed according to individual cultivar, there was a multitude of significant relationships detected (Table 6). Results indicated that there were significant negative correlations of Zx to hue and saturation; however, these relationships were only moderate in TifTuf, and weak in Latitude 36 and Tifway. Because Zx is present in relatively small quantities and is very sensitive to environment, it may be difficult if not impossible to establish a strong relationship between color values and Zx.

There were significantly ($P \leq 0.001$) moderate to strong relationships between the hue and saturation color parameters to TCh, Lu, Bc, and TCa for all three cultivars ranging from r = 0.62 to r = 0.91 across all sampling dates. The brightness color parameter was strongly significant ($P \leq 0.001$) and negatively correlated to the TCh, Lu, Bc, and TCa pigments (Table 6). Similar results were observed by Itle and Kabelka (2009). Negative correlations between brightness and pigments are expected because increases in pigmentation would increase color darkness and thereby decrease brightness. Similarly, because Zx concentrations increased while TCh decreased, there were negative correlations between Zx to hue and saturation while positive to brightness. This is because the loss of leaf pigment coloration would result in a brightening of the tissue color appearance. These results indicate that strong pigment to color value relationships exist among bermudagrass cultivars exposed to drought. The colors of plant pigments evaluated in this study range from green (TCh) to yellow (Lu, Zx) to orange (Bc). Because these pigments influenced each of the three color parameters independently, it may be possible in future research to develop a model that determines the individual contribution that each pigment has on turfgrass color as measured by digital image analysis.

In conclusion, changes in antioxidant pigments occurred in hybrid bermudagrass plants over a period of 28 d of drought stress. The TifTuf cultivar was able to maintain higher GC and TQ values longer than Latitude 36 and Tifway. The superior drought tolerance of TifTuf may be related to higher accumulations of Zx, Bc, and xanthophyll cycle activity at the later stages of drought stress, resulting in increased NPQ and ROS scavenging. Significant associations between antioxidant pigments and DGCI were observed with varying degrees of strength. Stronger correlations existed at the later stages of drought stress as leaves lost water content, and pigment concentrations attenuated to stress. Pigments of individual cultivars also correlated

moderately to strongly with hue, saturation, and brightness DIA color parameters throughout the study.

Carotenoids are important plant metabolites synthesized to help protect the photochemical machinery to drought and other stresses. In this study NPQ was not quantified; however, such information would be helpful in order to examine the effect that xanthophyll cycle-dependent thermal energy dissipation has on drought tolerance. Additionally, because Bc and other carotenoids have been implicated in the stabilization of cell membranes during drought, future research could be conducted to determine if increased accumulation of carotenoids can help prevent damage to cells by inhibiting membrane degradation. Due to the relationships established between color values and pigments concentrations, researchers may be able to develop models with which to help quickly predict antioxidant pigment content as an expeditious alternative to HPLC. Such methods could potentially be used in research pertaining to other stress such as cold, salt, or nutrient deficiencies. Furthermore, it may be possible to relate the color of each pigment to individual color parameters to help understand their contribution to overall turfgrass color. To further confirm the results of this study, comparisons of DIA color to other color measuring devices such as tristimulus colorimeters should be executed to determine if these relationships are able to be held true across devices. It would also be useful to repeat this type of experiment using other turfgrass species to determine if similar relationships can be made.

CHAPTER IV - CONCLUSIONS

It is of utmost importance to realize that the global water cycle can only provide a fraction of the Earth's 332.5 million cubic miles of water through readily available freshwater sources. This small, finite amount is needed to be shared among the 7+ billion humans that inhabit the planet for municipal, agricultural, and domestic use. As the population continues to increase, water will inevitably become even more of a scarce and precious resource. To exacerbate the issue, severe droughts occurring in various regions of the planet have led to increases in water shortages. Irrigation for turfgrass systems is often viewed as a wasteful use of water resources. As a result, ordinances have been placed in certain regions restricting the amount of water allowed for domestic purposes including turfgrass irrigation. Equally important, is the understanding that there are practical measures that can be taken to reduce irrigation water inputs in turfgrass systems. First, new irrigation systems are becoming available for more precise applications of water for turfgrass areas. Selecting the proper turfgrass for the specific site and knowing the water requirements of the turfgrass species being used is something that all homeowners and turf managers should become more aware of to avoid unnecessary applications of water. Although typically considered an amenity, turfgrasses can provide a wide range of environmental and economic benefits that warrant their use in a variety modern day landscapes. Additionally, they offer a safe and reliable playing surface for athletes in a range of sporting activities. Therefore, strong efforts should be made to develop new drought resistant cultivars of turfgrass.

Turfgrasses have developed a variety of drought resistant mechanisms including escape, avoidance, and tolerance. New commercially available cultivars of hybrid bermudagrass such as TifTuf and Latitude 36 have been developed by turfgrass breeders that possess improved stress

resistance to various factors. However, the means by which these newer cultivars have adapted to drought are largely uninvestigated.

In the first part of this research, hybrid bermudagrass cultivars were compared for their rooting and water use characteristics. It was found that the most drought resistant cultivar, TifTuf, was characterized by greater root weight, in addition to higher root surface area and root volume at deeper soil depths. Roots of TifTuf were well-distributed throughout the soil profile compared to the other two cultivars resulting in better turfgrass quality at later stages of drought stress.

The second part of this research was intended to characterize differential responses of antioxidant pigments among cultivars as drought progressed, in addition to investigating the relationship of antioxidant pigments to turfgrass color. The most drought resistant cultivar, TifTuf, was characterized by having higher concentrations of zeaxanthin and increased activity of the xanthophyll cycle at the later stages of drought stress. Such increases can result in greater photoprotection, thus improve drought stress tolerance. TifTuf also yielded increased concentrations of β-carotene as drought progressed, whereas other cultivars decreased β-carotene concentrations. This may have led to the improved stress tolerance of TifTuf by means of an increased xanthophyll cycle pool allowing for direct scavenging of reactive oxygen species. Xanthophylls and other carotenoids also have roles in membrane stabilization during drought stress, suggesting that cultivars like TifTuf with higher carotenoid concentrations may be better able to withstand desiccation through improved membrane integrity.

Antioxidant pigments in addition to chlorophyll were significantly associated with DGCI, with stronger associations detected at the later stages of drought. Pigments of individual cultivars were also correlated to the hue, saturation, and brightness color parameters analyzed via

digital image analysis (DIA). Researchers may be able to use the information gained from these relationships to further develop regression models to help predict antioxidant content in drought tolerant turfgrass species. Developing the use of DIA color analysis as a faster way to estimate carotenoid content would provide researchers with an expeditious alternative to HPLC extractions.

It is known that plants can possess more than just one of the three drought resistance mechanisms of escape, avoidance, and tolerance. The results of this research have shown that avoidance mechanisms such as more robust rooting and tolerance mechanisms like increased antioxidant pigment concentrations are both likely to improve drought stress resistance in cultivars like TifTuf.

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APPENDIX

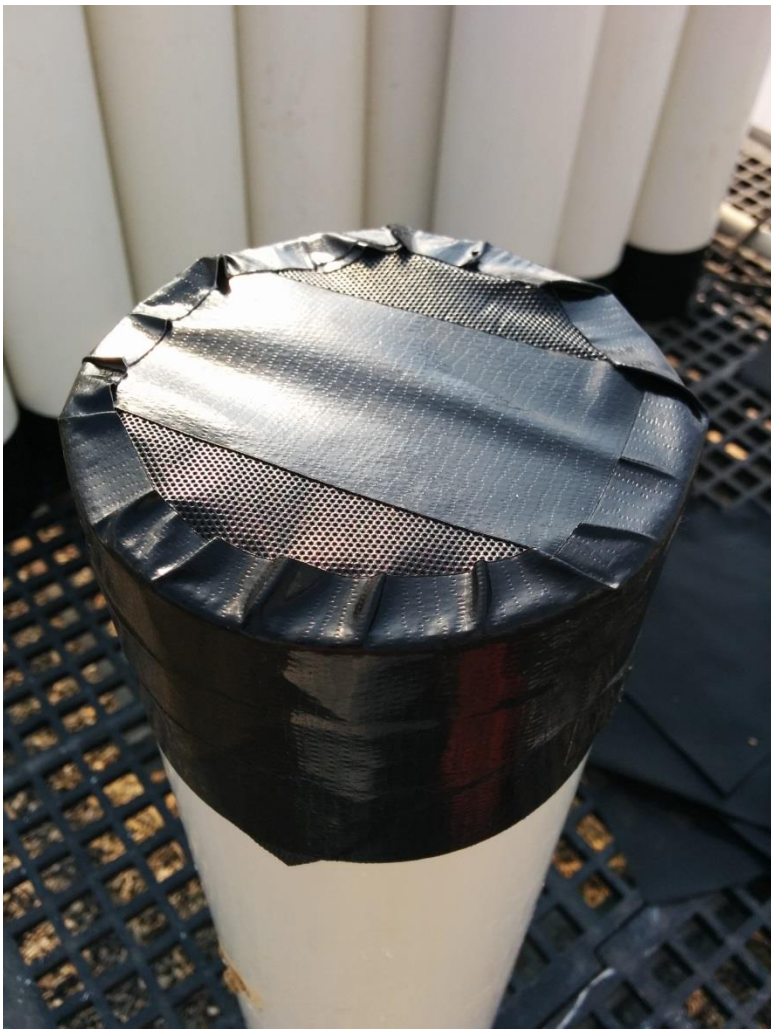

Figure 1. Bottom view of completed lysimeter with landscape fabric fastened with outdoor grade duct tape to one end. Lysimeter constructed from 45-cm length of 10.3-cm (inside diameter) sewer pipe.

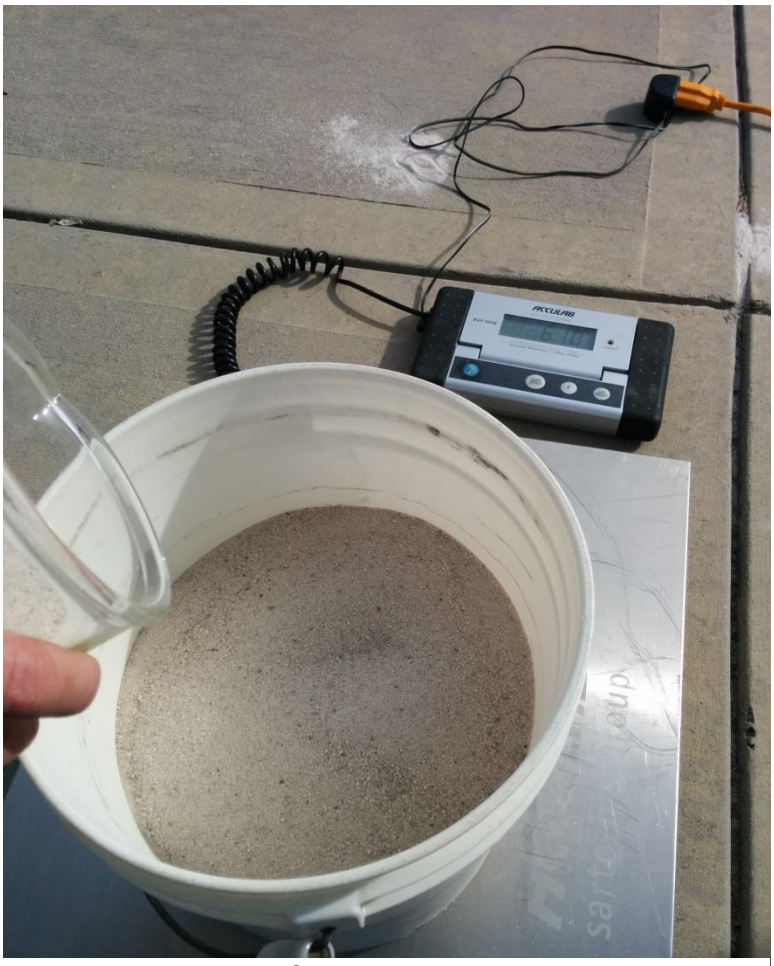

Figure 2. Weighing of dry sand to be added to the lysimeters. Each lysimeter was filled with 6.18 kg of sand to a bulk density of 1.57 g cm^{-3}.

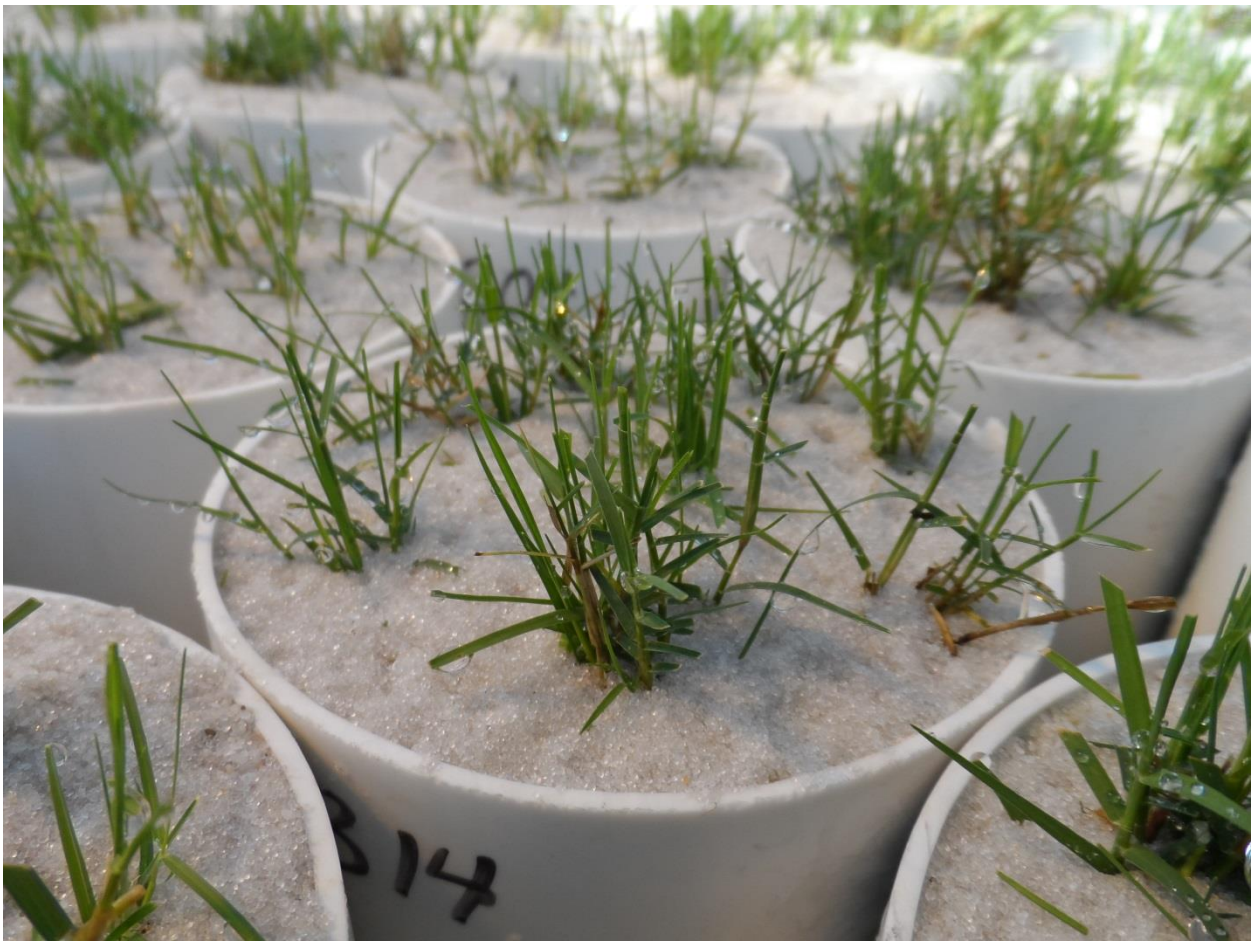

Figure 3. Propagation of eight evenly spaced hybrid bermudagrass plants of three tillers each into lysimeters in a glasshouse environment at the University of Tennessee Institute of Agriculture campus on 26 May 2015. Glasshouse conditions were set to an 18 h photoperiod under high pressure sodium grow lights with 35 / 21 °C daytime / nighttime temperature.

Figure 4. Rainout box constructed using plywood walls to a height similar to that of the lysimeters with installed weather station and light meter (red arrows). A hinged clear lid was attached to allow light to enter while the lid was closed during periods of rain.

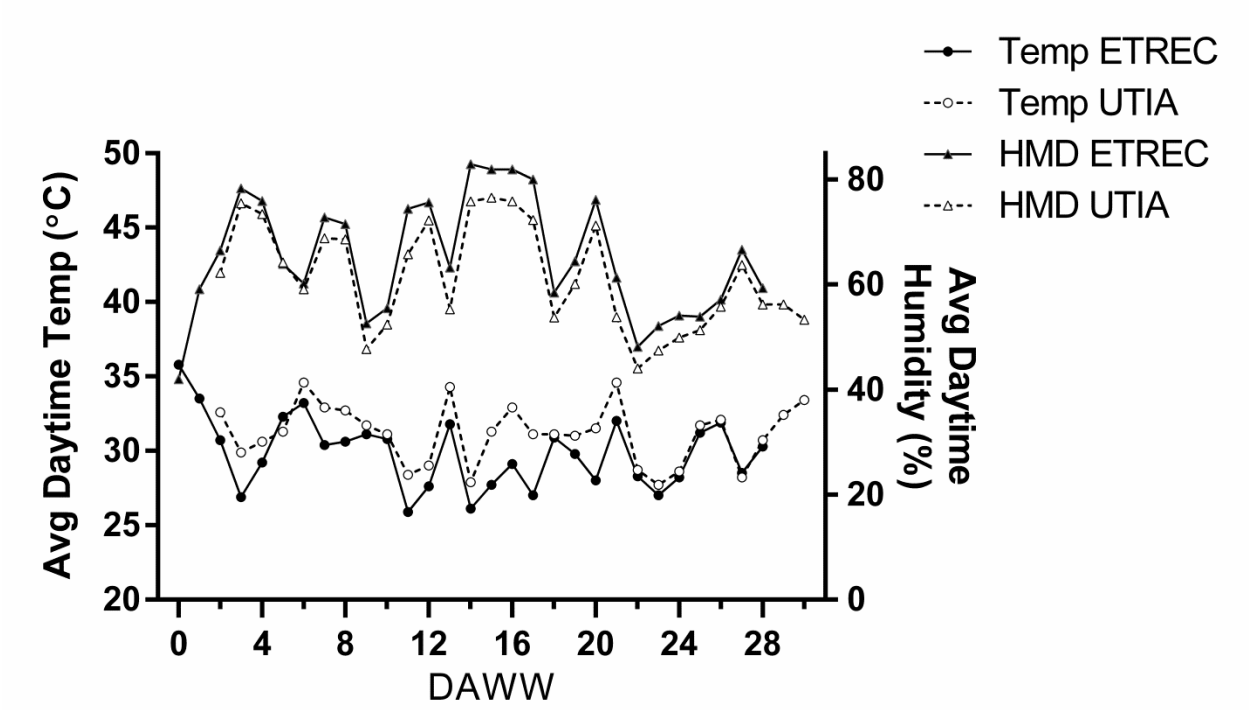

Figure 5. Average daytime temperature (Temp) and percent humidity (HMD) across 28 d after withholding water (DAWW) at two locations in East Tennessee. Data recorded hourly between 0800 and 2000 h each day beginning on 3 Aug 2015 at the East Tennessee Research and Education Center (ETREC) and on 5 Aug 2015 at the University of Tennessee Institute of Agriculture (UTIA) campus.

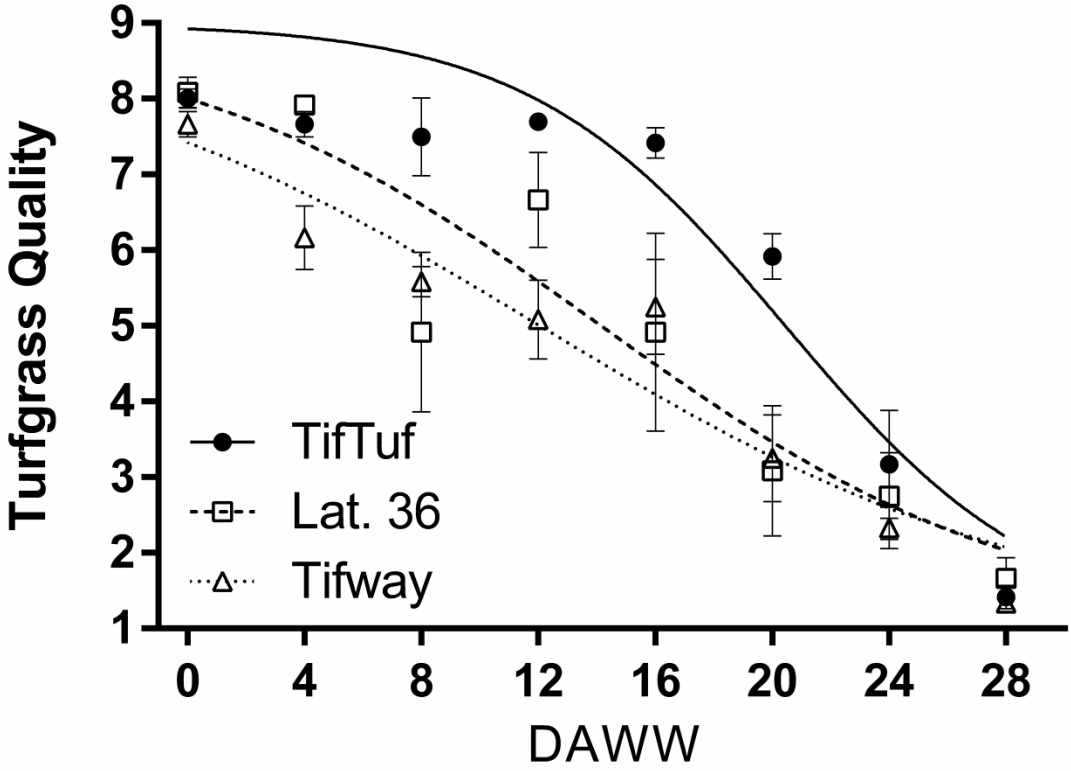

Figure 6. Nonlinear regression analysis of pooled means for turfgrass quality (1 to 9 scale, 1 = poorest quality, 9 = ideal turf, 6 = minimum acceptable turf quality) of three cultivars of hybrid bermudagrass from 0 to 28 d after withholding water (DAWW). Symbols represent pooled means collected on 4 d intervals at approximately 1100 h in August of 2015 at two locations in East Tennessee. Errors bars represent the standard error of the mean for each rating date.

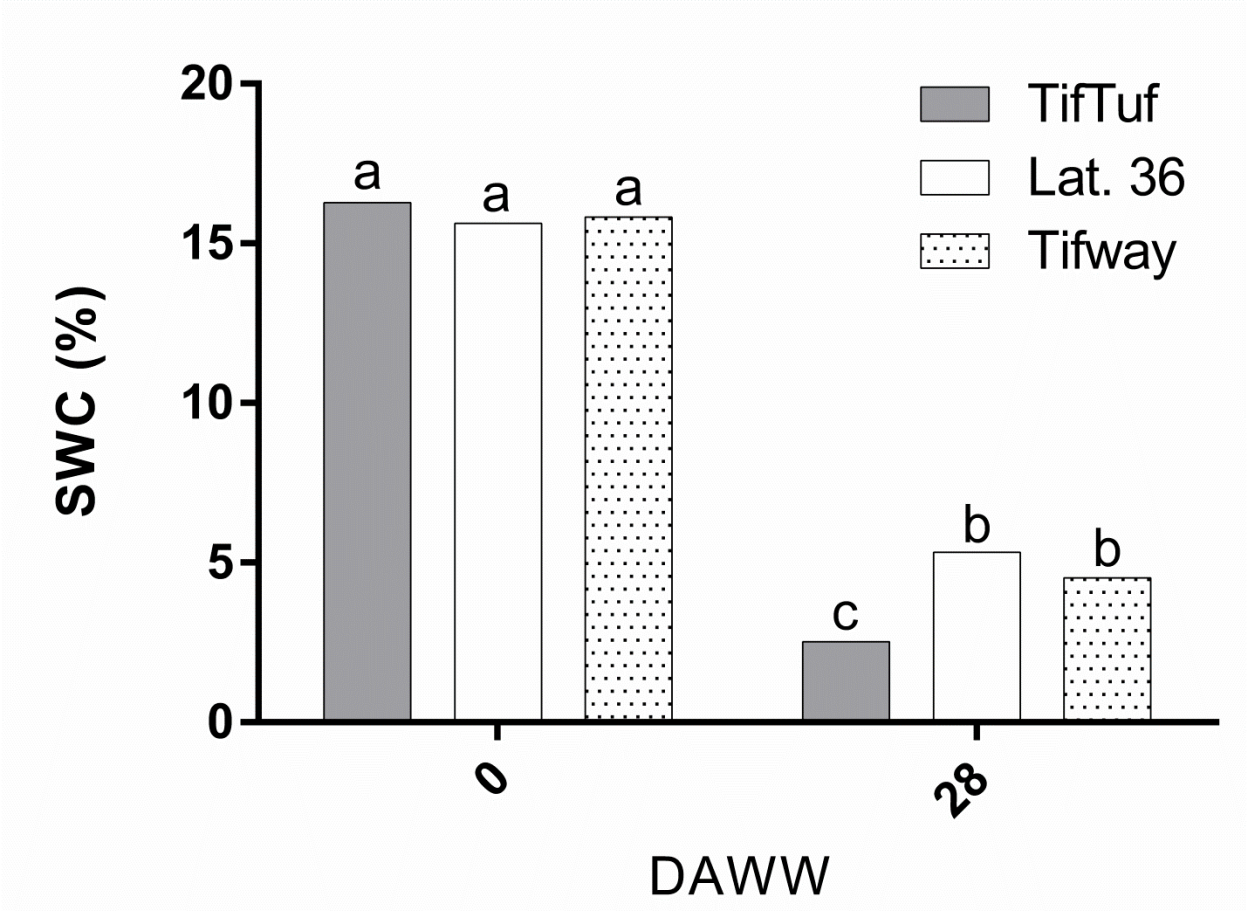

Figure 7. Mean percent soil water content (SWC) measured at 0 and 28 d after withholding water (DAWW) for three hybrid bermudagrass cultivars. Means are pooled across two locations in East Tennessee in August of 2015. Means labeled with the same letter do not significantly differ according to Fisher's protected LSD the *P* < 0.05 level of probability.

Table 1. Mean root and rhizome dry mass and whole plant root length for each cultivar at three soil depths at the end of 28 d after withholding water. Means are pooled across two locations in East Tennessee in August of 2015. Total dry root mass is the sum of all three depth categories, and rhizome dry weights are means of rhizomes harvested from individual cultivars. Means followed by the same letter within each column do not significantly differ according to Fisher's protected LSD at the $P \leq 0.05$ level of probability.

Cultivar	Root and Rhizome Dry Mass (g)					Root Length (cm)
	Soil Depth (cm)					
	0-15	15-30	30-45	Total	Rhizomes	
TifTuf	1.07	0.56 a	0.18 a	1.82 a	2.56 a	42.8
Latitude 36	1.04	0.27 b	0.02 b	1.33 b	1.62 b	34.8
Tifway	1.00	0.32 b	0.03 b	1.35 b	2.02 ab	35.8
P value summary	NS	*	**	*	*	NS

*** and **, significant at $P \leq 0.05$ and 0.01, respectively. NS, not significant**

Table 2. WinRHIZO analyses for Mean total root length (TRL), surface area (SA), average diameter (AD), volume (RV), and root length density (RLD) for three hybrid bermudagrass cultivars at three soil depths at the end of 28 d of drought. Means are pooled across two locations in East Tennessee in August of 2015. Total means are the sum of all three depth categories. Means followed by the same letter within each column do not significantly differ according to Fisher's protected LSD at the $P \leq 0.05$ level of probability.

Cultivar	Soil Depth (cm)	TRL (cm)	SA (cm^2)	AD (mm)	RV (cm^3)	RLD ($m\ m^{-3}$)
TifTuf	0-15	17910	1040	0.18	4.81	1493
Latitude 36		15140	864	0.18	3.96	1262
Tifway		17330	957	0.18	4.23	1444
P value Summary		NS	NS	NS	NS	NS
TifTuf	15-30	5486	474	0.27	3.29	457.2
Latitude 36		3496	304	0.26	2.16	291.3
Tifway		3583	295	0.27	1.96	298.6
P value Summary		NS	NS	NS	NS	NS
TifTuf	30-45	181	165 a	0.32	1.21 a	150.5
Latitude 36		334	25.9 b	0.13	0.16 b	27.85
Tifway		463	39.5 b	0.22	0.27 b	38.57
P value Summary		NS	*	NS	*	NS
TifTuf	Total	25210	1675 a	0.26	9.32 a	2101
Latitude 36		18970	1195 b	0.19	6.28 b	1581
Tifway		21380	1292 ab	0.22	6.46 b	1782
P value Summary		NS	*	NS	*	NS

***, significant at $P \leq 0.05$. NS, Not significant**

[1]

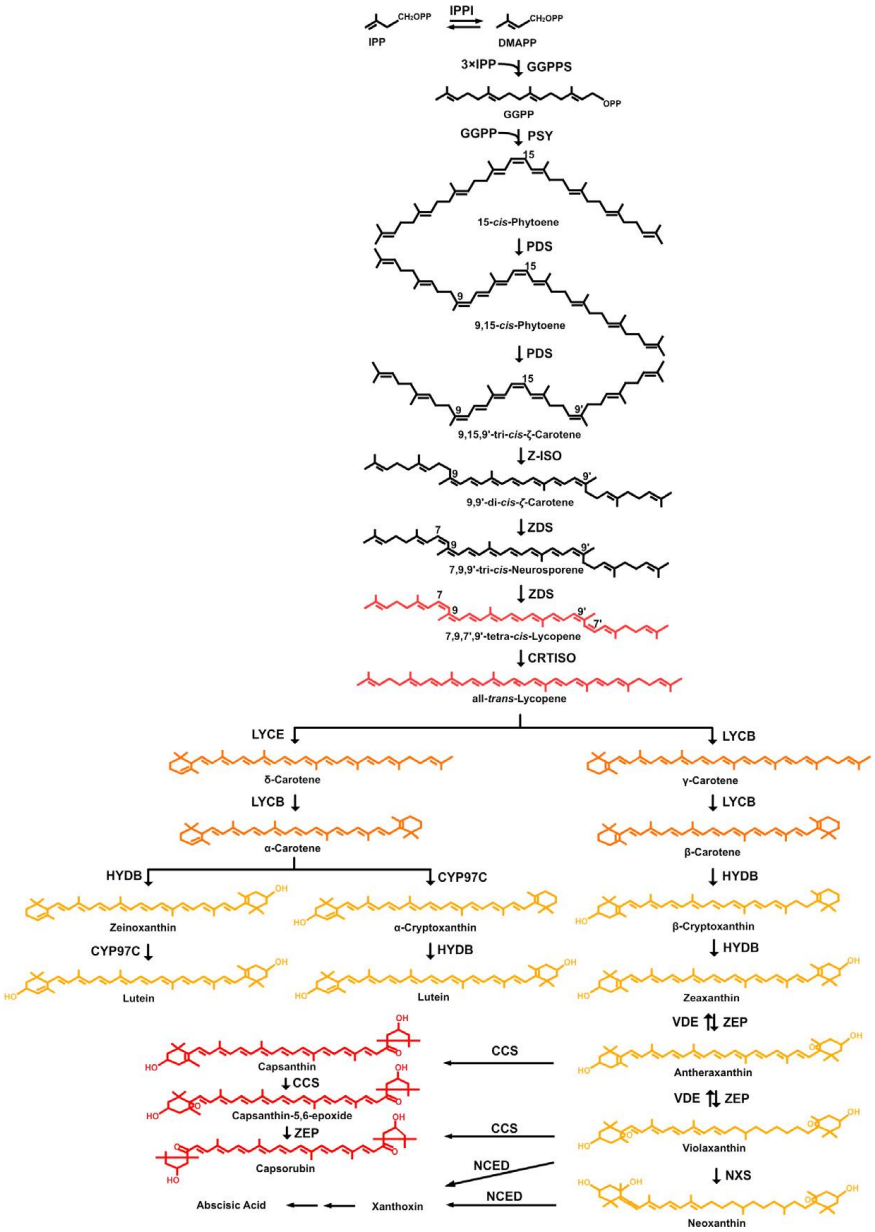

Figure 8. Carotenoid biosynthetic pathway in plants. Abbreviations: IPP, isopentenyl diphosphate; IPPI, isopentenyl diphosphate isomerase; DMAPP, dimethylallyl diphosphate; GGPP, geranygeranyl diphosphate; GGPPS, GGPP synthase; PSY, phytoene synthase; PDS, phytoene desaturase; Z-ISO, ζ-carotene isomerase; ZDS, ζ-carotene desaturase; CRTISO, carotenoid isomerase; LYCB, lycopene β-cyclase; LYCE, lycopene ε-cyclase; CCS, capsanthin-capsorubin synthase; CYP97C, carotene ε-ring hydroxylase; HYDB, β-carotene hydroxylase; ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; NXS, neoxanthin synthase; NCED, 9-cis-epoxycarotenoid dioxygenase.

[1] Reprinted from Archives of Biochemistry and Biophysics, Vol. 504, Zhu et al., *The Regulation of Carotenoid Pigmentation in Flowers*, p. 132-141., Copyright (2010), with permission from Elsevier.

Figure 9. Illuminated box used for digital image analysis. Plants were placed under two compact fluorescent light bulbs which provided consistent lighting conditions among images. The box was enclosed on all sides to prevent outside light from entering. Images were acquired using a digital camera placed directly above the lysimeter in a darkened lab on the University of Tennessee Institute of Agriculture campus.

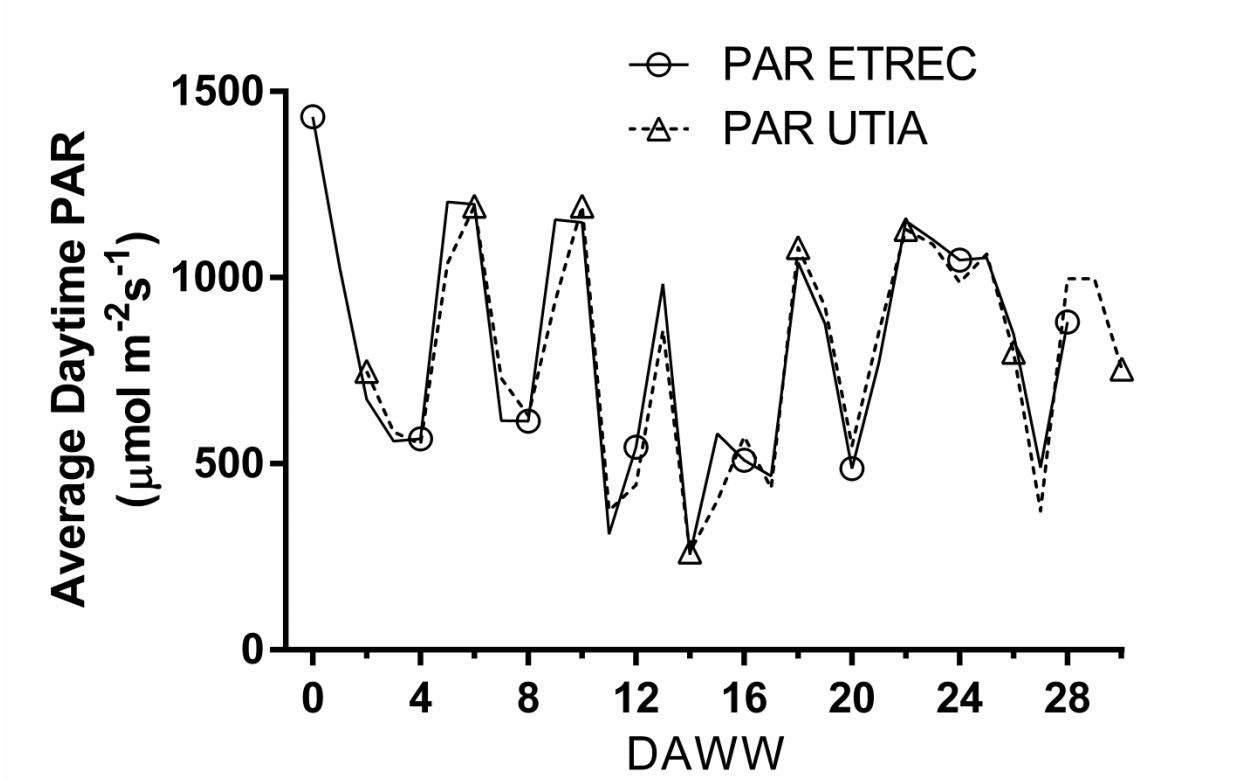

Figure 10. Average daytime photosynthetically active radiation (PAR) across 28 d after withholding water (DAWW) at two locations in East Tennessee. PAR measured hourly at each location between 0800 and 2000 h beginning on 3 Aug 2015 at the East Tennessee Research and Education Center and on 5 Aug 2015 at the University of Tennessee Institute of Agriculture (UTIA) campus. Symbols along each line represent the average PAR in μmol $m^{-2}s^{-1}$ for each independent sampling date at ETREC (open circles) and at UTIA (open triangles).

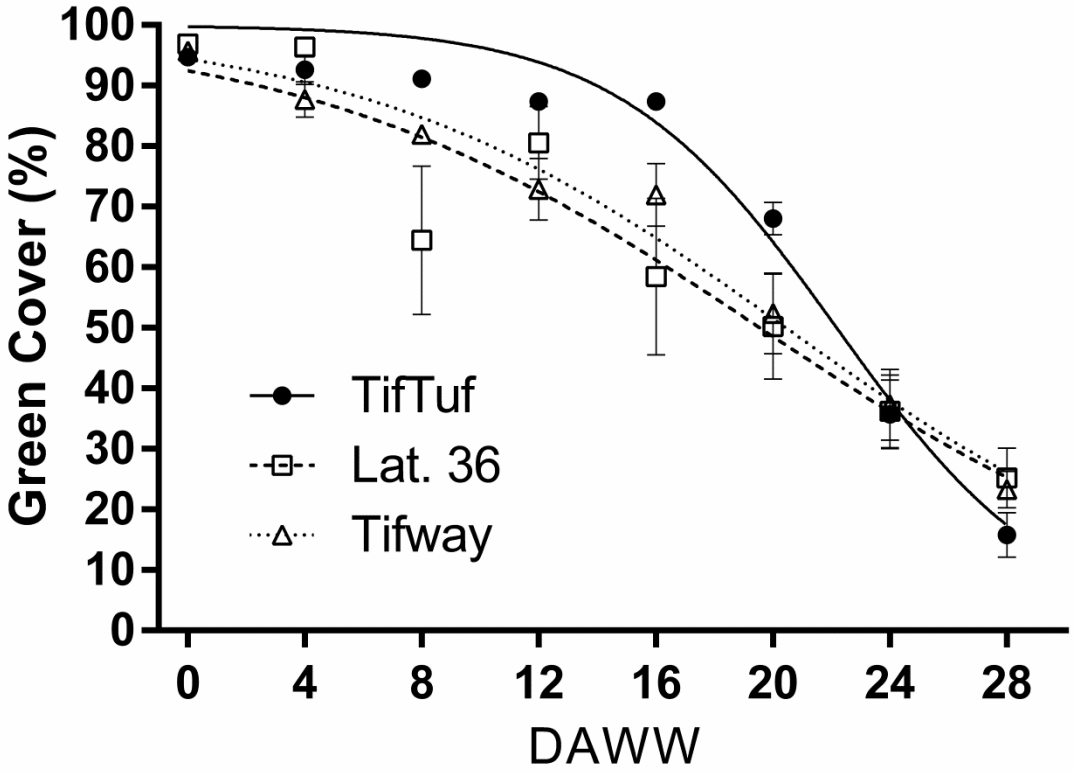

Figure 11. Nonlinear regression analysis for percent green turfgrass cover (measured using digital image analysis) of three hybrid bermudagrass cultivars across 28 d after withholding water (DAWW) at two locations in East Tennessee in August of 2015. Symbols represent pooled means collected on 4 d intervals at approximately 1200 h in August of 2015 at two locations in East Tennessee. Errors bars represent the standard error of three replications per location within each individual sampling date.

Table 3. Results of nonlinear regression analysis to determine the average number of days required for three hybrid bermudagrass cultivars to reach a drought response of 50, 55, and 75% green cover across 28 d of drought stress. Means of three replications are pooled across two locations in East Tennessee in August of 2015.

Cultivar	GC_x[†]	$Days_x$[‡]	95% C.I.[§]		Slope[¶]	R^2
			Lower	Upper		
TifTuf		22.2	21.5	22.8	-0.12	0.92
Latitiude 36	GC_{50}	19.6	17.2	22.0	-0.06	0.61
Tifway		20.4	19.2	21.7	-0.06	0.84
TifTuf		21.4*	20.8	22.1	-0.12	0.92
Latitiude 36	GC_{55}	18.0	15.7	20.3	-0.06	0.61
Tifway		19.0	17.8	20.2	-0.06	0.84
TifTuf		18.1*	17.2	19.0	-0.12	0.92
Latitiude 36	GC_{75}	11.0	7.70	14.3	-0.06	0.61
Tifway		12.5	10.8	14.2	-0.06	0.84

* Significantly different at $P \leq 0.05$.

[†] Percent response (50, 55, 75%) of green color (GC) to drought stress.

[‡] Average number of days to reach GC_x.

[§] 95% confident interval of $Time_x$. Overlapping C.I. indicates that $Time_x$ does not differ at the $P \leq 0.05$ level of probability.

[¶] Slope of nonlinear regression line at GC_x.

Table 4. Results of nonlinear regression analysis to determine the number of days for three hybrid bermudagrass cultivars to reach the minimum turfgrass quality rating (TQ = 6) across 28 d of drought stress. Means of three replications are pooled across two locations in East Tennessee in August of 2015.

Cultivar	TQ_6[†]	95% C.I.[‡]		Slope[§]	R^2
		Lower	Upper		
TifTuf	17*	16.0	18.8	-0.10	0.79
Latitiude 36	9.0	5.90	12.3	-0.06	0.57
Tifway	6.0	3.83	8.38	-0.05	0.76

* Significantly different at $P \leq 0.05$.

[†] Number of days to reach TQ_6.

[‡] 95% confident interval of TQ_6. Overlapping C.I. indicates that TQ_6 does not differ at the $P \leq 0.05$ level of probability.

[§] Slope of nonlinear regression line.

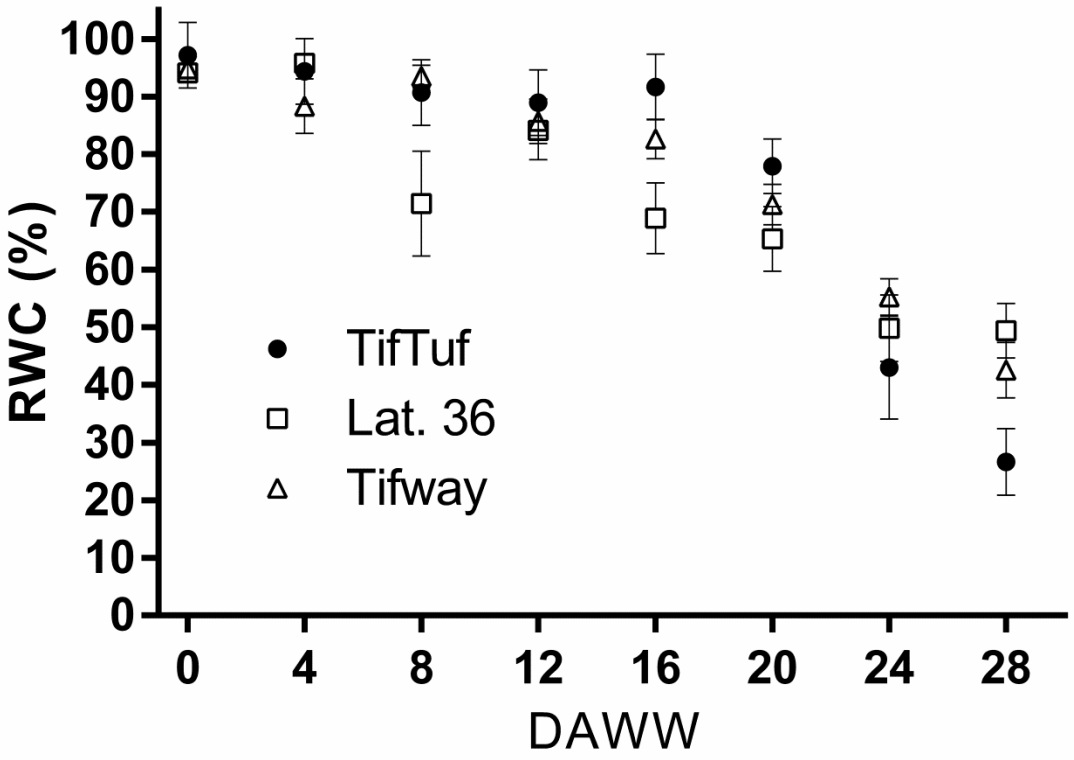

Figure 12. Mean percent relative water content (RWC) of three hybrid bermudagrass cultivars across 28 d after withholding water (DAWW). Symbols represent pooled means collected on 4 d intervals at approximately 1200 h in August of 2015 at two locations in East Tennessee. Errors bars represent the standard error of the mean of three replications per location within each individual sampling date.

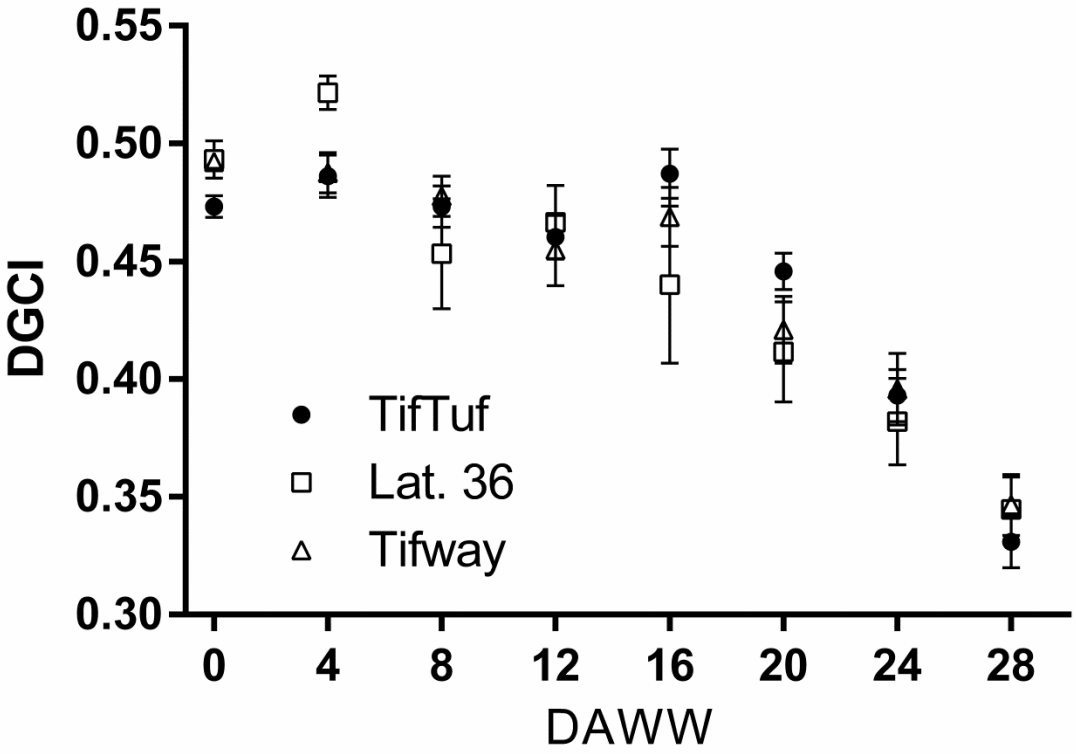

Figure 13. Mean dark green color index (DGCI) results from digital image analysis of three hybrid bermudagrass cultivars across 28 d after withholding irrigation (DAWW). Symbols represent pooled means collected on 4 d intervals at approximately 1200 h in August of 2015 at two locations in East Tennessee. Errors bars represent the standard error of the mean of three replications per location within each individual sampling date.

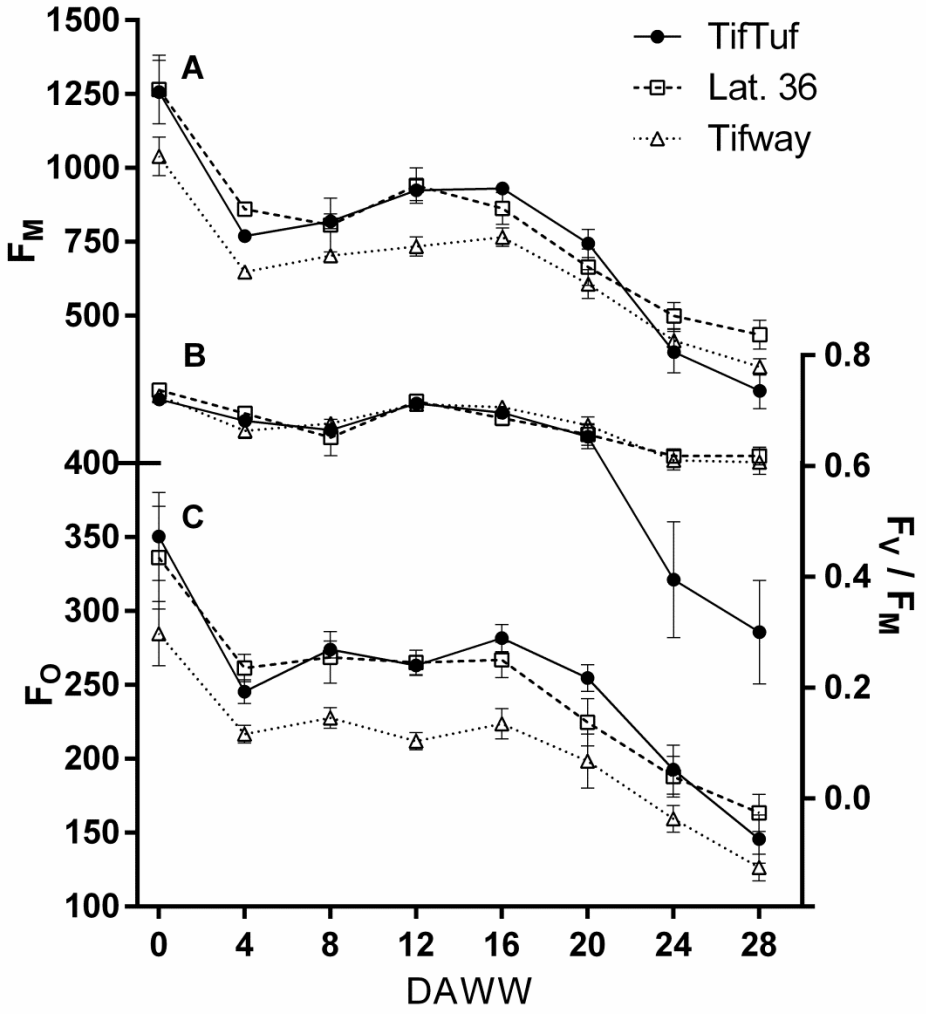

Figure 14. Fluorescence data collected using a pulse-amplitude-modulated (PAM) fluorimeter for three hybrid bermudagrass cultivars across 28 d after withholding water (DAWW). Symbols represent pooled means collected on 4 d intervals at approximately 1200 h in August of 2015 at two locations in East Tennessee. Errors bars represent the standard error of the mean of three replications per location within each individual sampling date. F_M = Maximal fluorescence (A); F_0 = Minimal fluorescence (C); F_V/F_M = maximum quantum yield of photosynthesis (B).

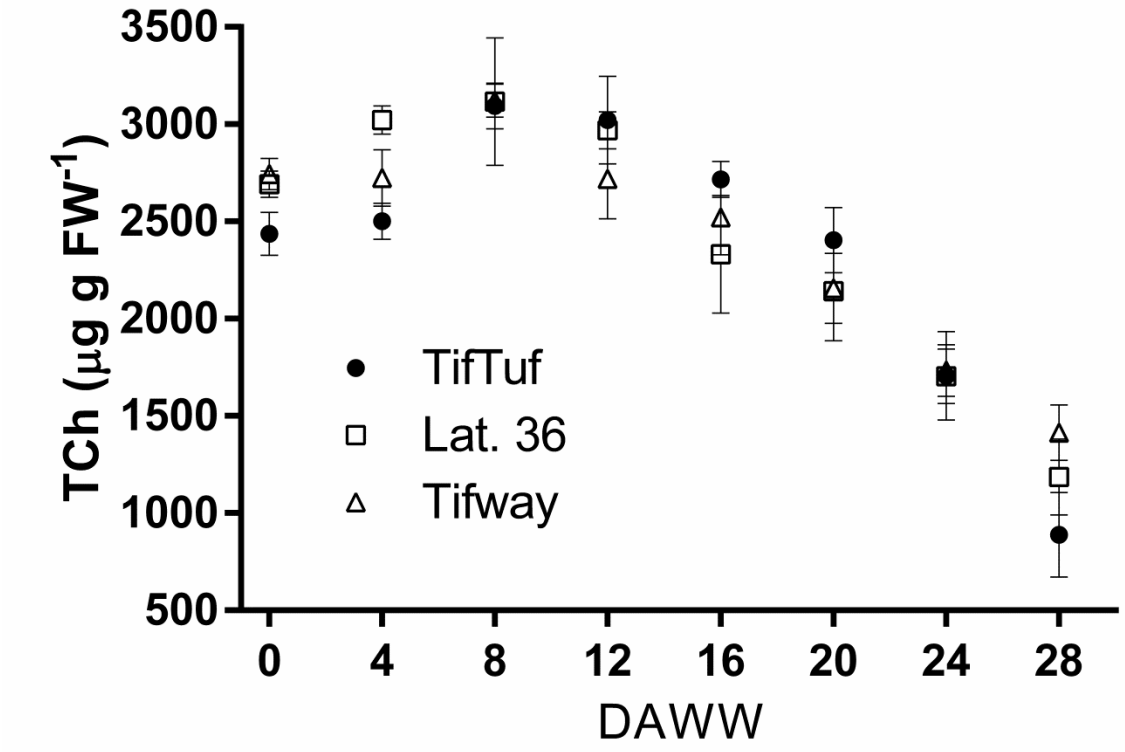

Figure 15. Day-by-cultivar interaction for mean total chlorophyll concentration measured from a 0.25 g fresh weight (FW) sample of hybrid bermudagrass leaf tissue using high performance liquid chromatography. Symbols represent pooled means collected on 4 d intervals across 28 days after withholding water (DAWW) sampled at approximately 1200 h in August of 2015 at two locations in East Tennessee. Errors bars represent the standard error of the mean of three replications per location within each individual sampling date.

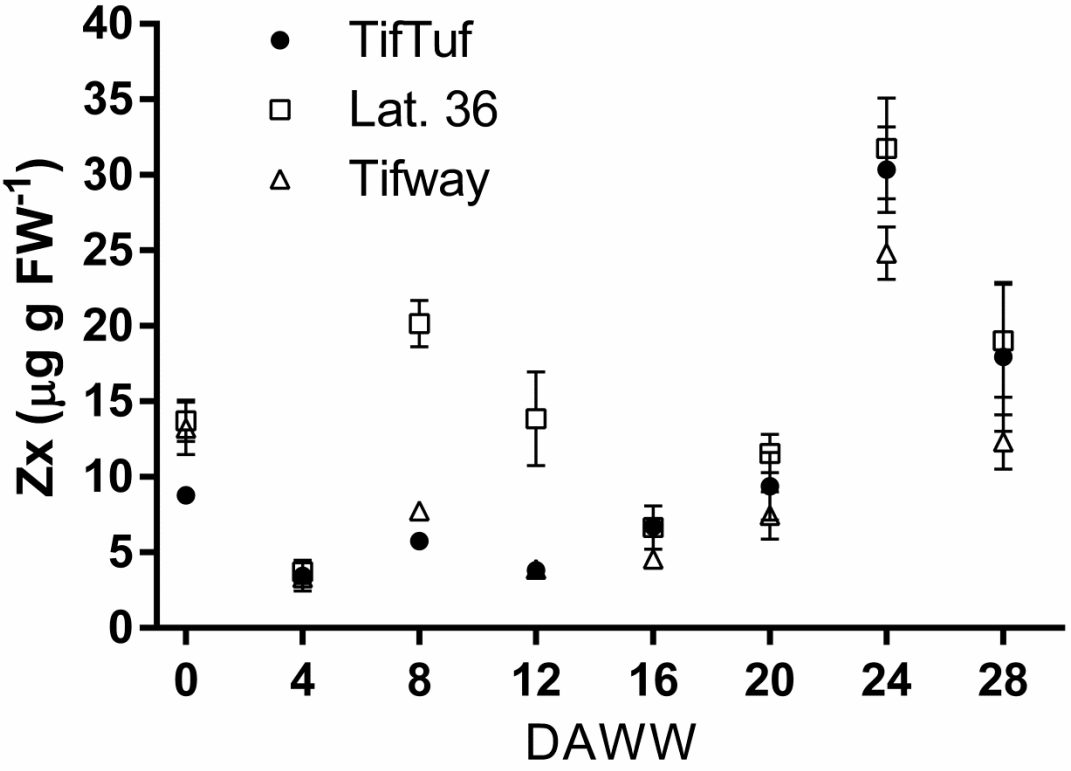

Figure 16. Day-by-cultivar interaction for mean total zeaxanthin (Zx) concentration measured from a 0.25 g fresh weight (FW) sample of hybrid bermudagrass leaf tissue using high performance liquid chromatography. Samples collected at 4 d intervals across 28 d after withholding water (DAWW) beginning on 3 Aug 2015 at the East Tennessee Research and Education Center. Error bars are standard errors of the means of three replications within each sampling date.

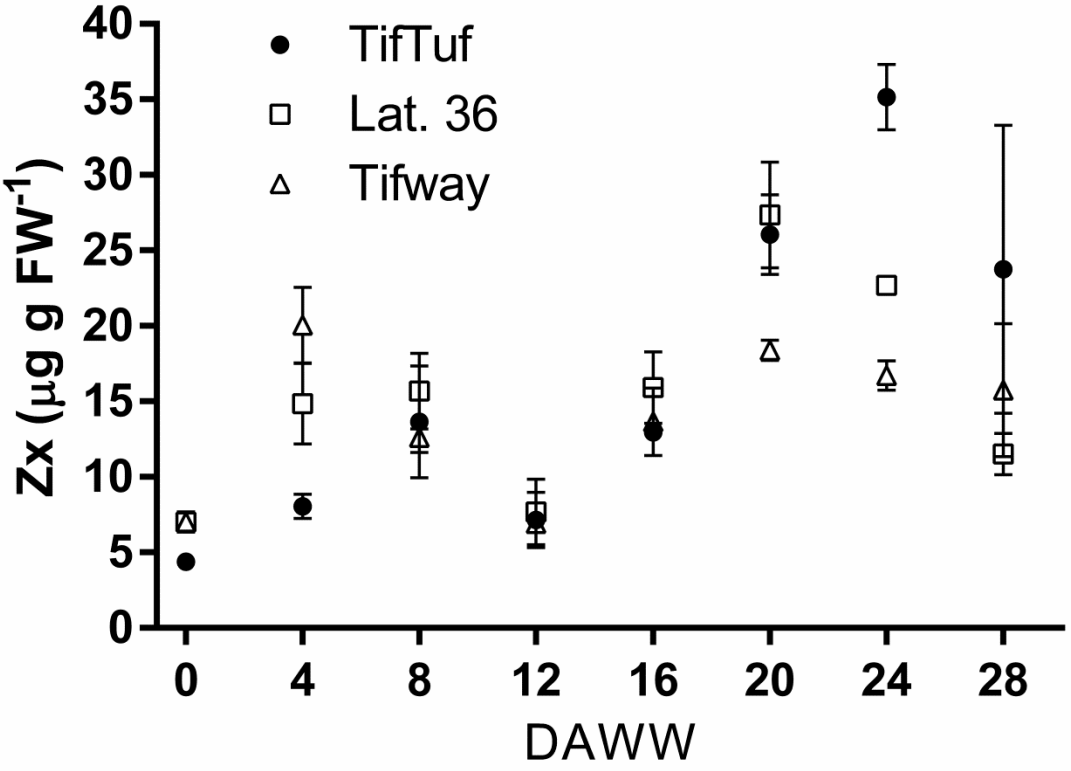

Figure 17. Day-by-cultivar interaction for mean total zeaxanthin (Zx) concentration measured from a 0.25 g fresh weight (FW) sample of hybrid bermudagrass leaf tissue using high performance liquid chromatography. Samples collected at 4 d intervals across 28 d after withholding water (DAWW) beginning on 5 Aug 2015 at the University of Tennessee Institute of Agriculture campus. Error bars are standard errors of the means of three replications within each sampling date.

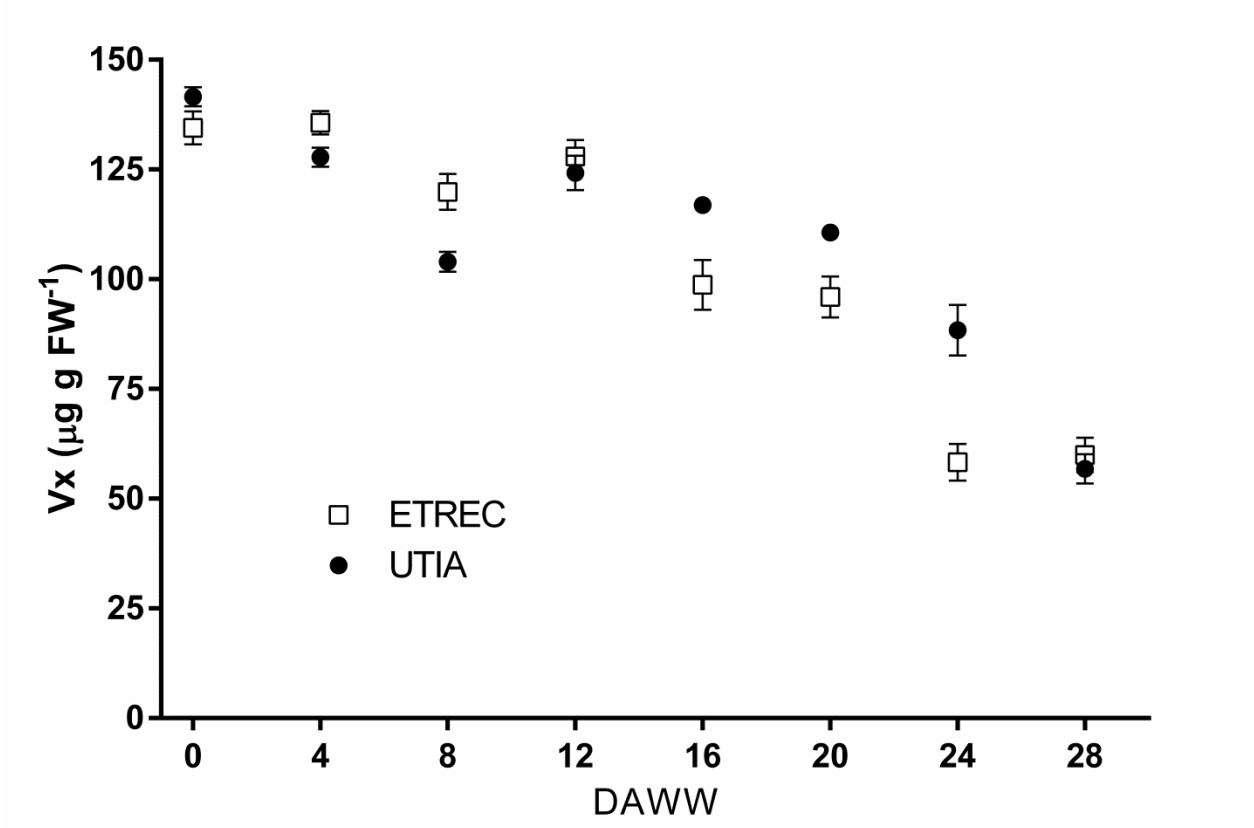

Figure 18. Day-by-location interaction for mean total violaxanthin (Vx) concentration measured from a 0.25 g fresh weight (FW) sample of hybrid bermudagrass leaf tissue beginning on 3 Aug 2015 at the East Tennessee Research and Education Center (ETREC) and on 5 Aug 2015 at the University of Tennessee Institute of Agriculture (UTIA). Samples collected at 4 d intervals across 28 d after withholding water (DAWW) and quantified using high performance liquid chromatography. Error bars are standard errors of the means for all cultivars pooled together within each sampling date.

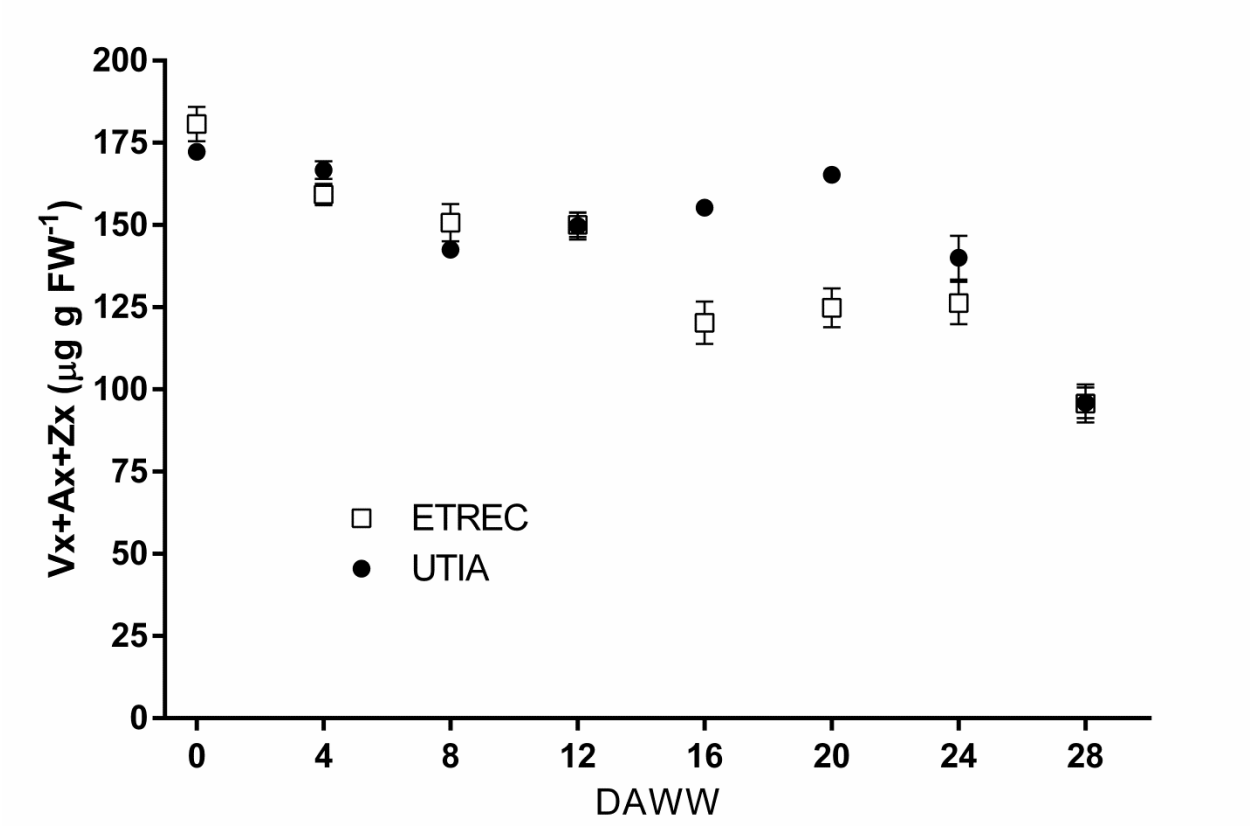

Figure 19. Day-by-location interaction for mean total Violaxanthin (Vx), Antheraxanthin (Ax), and zeaxanthin (Zx) concentration measured from a 0.25 g fresh weight (FW) sample of hybrid bermudagrass leaf tissue beginning on 3 Aug 2015 at the East Tennessee Research and Education Center (ETREC) and on 5 Aug 2015 at the University of Tennessee Institute of Agriculture (UTIA). Samples collected at 4 d intervals across 28 d after withholding water (DAWW) and quantified using high performance liquid chromatography. Error bars are standard errors of the means for all cultivars pooled together within each sampling date.

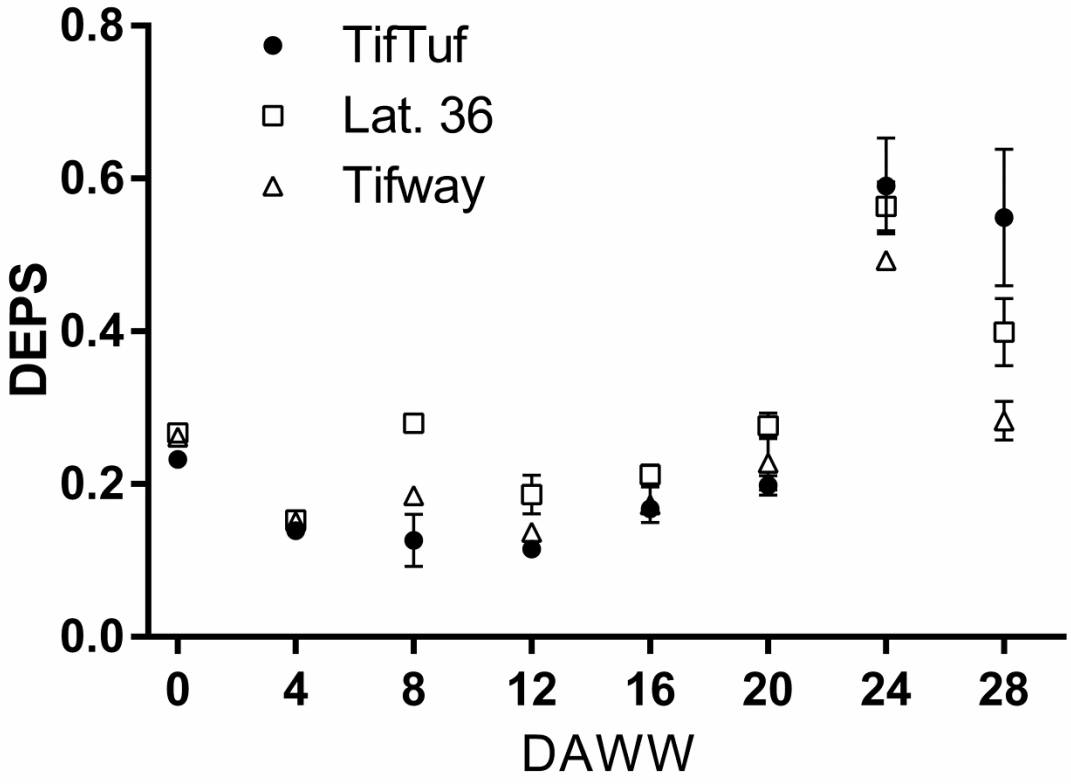

Figure 20. Day-by-cultivar interaction for the mean de-epoxidation state of the xanthophyll cycle (DEPS) at the East Tennessee Research and Education Center. Symbols represent pooled means of fresh leaf tissue samples collected on 4 d intervals across 28 d after withholding water (DAWW) sampled at approximately 1200 h beginning on 3 Aug 2015. Errors bars represent the standard error of the mean of three replications within each individual sampling date.

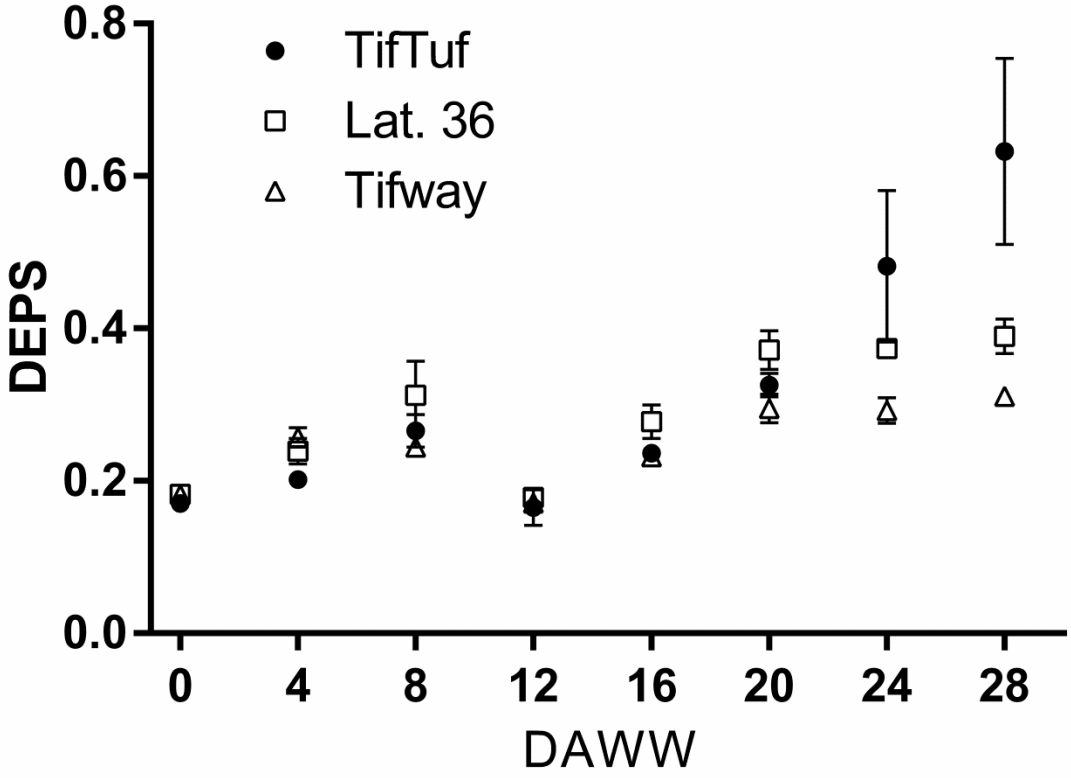

Figure 21. Day-by-cultivar interaction for the mean de-epoxidation state of the xanthophyll cycle (DEPS) at the University of Tennessee Institute of Agriculture campus. Symbols represent pooled means of fresh leaf tissue samples collected on 4 d intervals across 28 d after withholding water (DAWW) sampled at approximately 1200 h beginning on 5 Aug 2015. Errors bars represent the standard error of the mean of three replications within each individual sampling date.

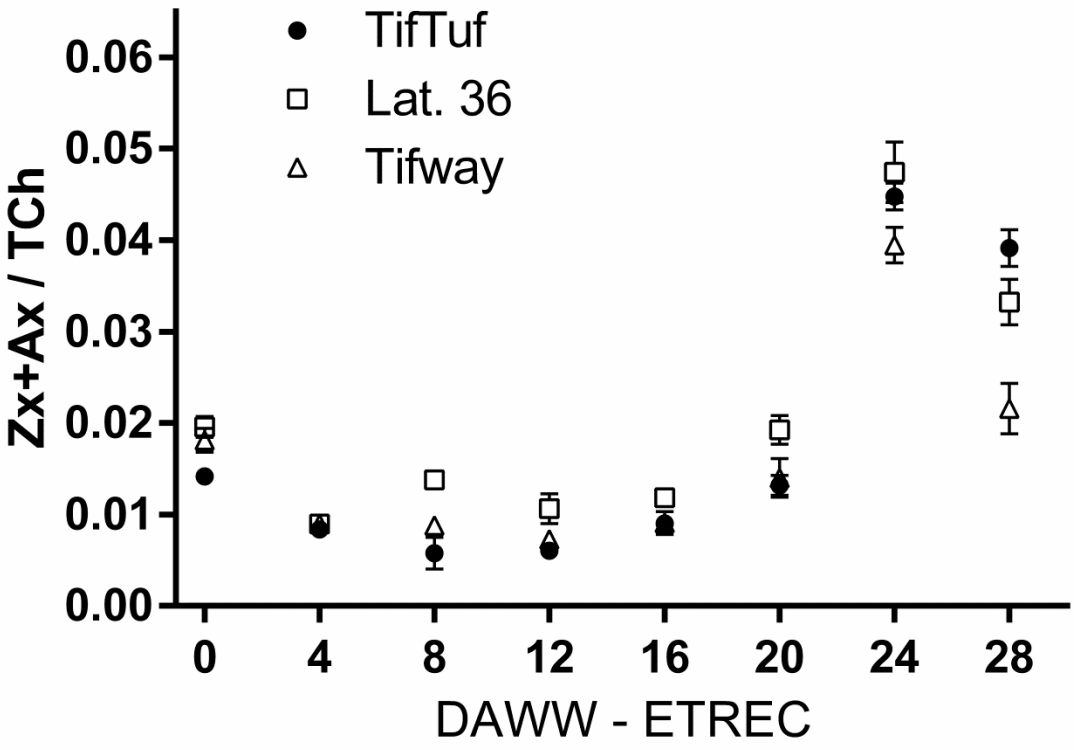

Figure 22. Day-by-cultivar interaction for the ratio of total zeaxanthin (Zx) plus antheraxanthin (Ax) to total chlorophyll (TCh). Pigments quantified using high performance liquid chromatography. Samples of fresh leaf tissue collected at 4 d intervals across 28 d after withholding water (DAWW) beginning on 3 Aug 2015 at the East Tennessee Research and Education Center (ETREC). Error bars are standard errors of the means of three replications within each sampling date.

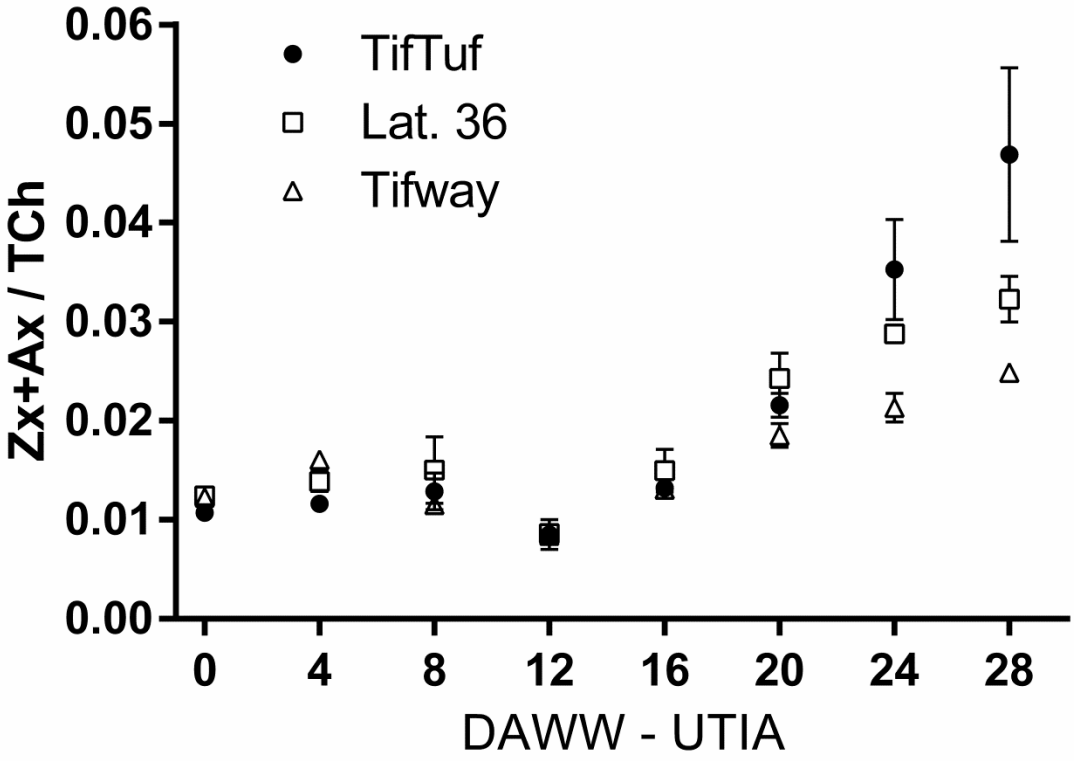

Figure 23. Day-by-cultivar interaction for the ratio of total zeaxanthin (Zx) plus antheraxanthin (Ax) to total chlorophyll (TCh). Pigments quantified using high performance liquid chromatography. Samples of fresh leaf tissue collected at 4 d intervals across 28 d after withholding water (DAWW) beginning on 5 Aug 2015 at the University of Tennessee Institute of Agriculture campus. Error bars are standard errors of the means of three replications within each sampling date.

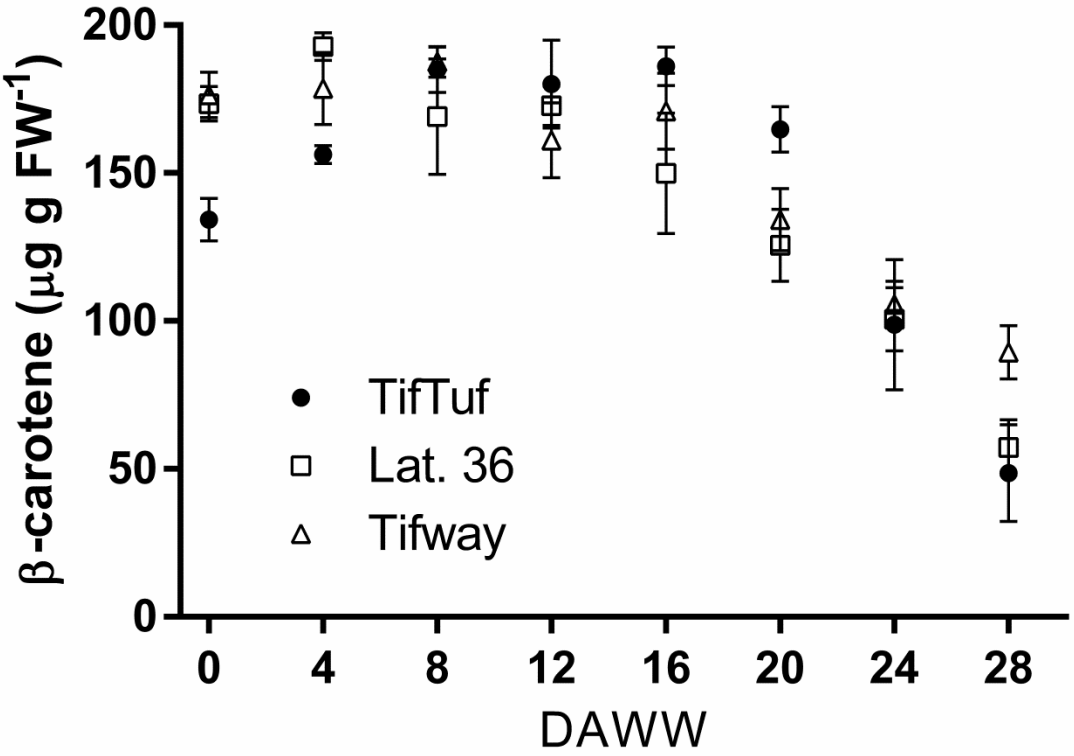

Figure 24. Day-by-cultivar interaction for mean β-carotene concentration measured from a 0.25 g fresh weight (FW) sample of hybrid bermudagrass leaf tissue using high performance liquid chromatography. Symbols represent pooled means collected on 4 d intervals across 28 d after withholding water (DAWW) sampled at approximately 1200 h in August of 2015 at two locations in East Tennessee. Errors bars represent the standard error of the mean of three replications per location within each individual sampling date.

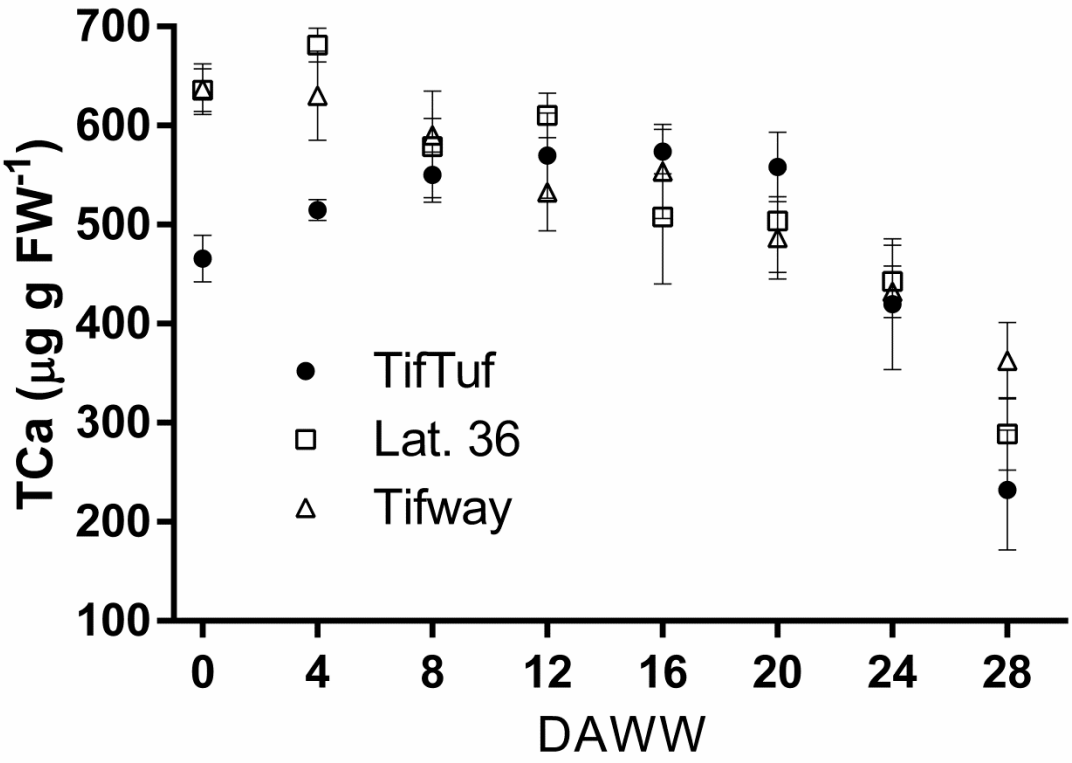

Figure 25. Day-by-cultivar interaction for mean total carotenoid concentration measured from a 0.25 g fresh weight (FW) sample of hybrid bermudagrass leaf tissue using high performance liquid chromatography. Symbols represent pooled means collected on 4 d intervals across 28 d after withholding water (DAWW) sampled at approximately 1200 h in August of 2015 at two locations in East Tennessee. Errors bars represent the standard error of the mean of three replications per location within each individual sampling date.

Table 5. Pearson correlation coefficients (*r*) between dark green color indices from digital image analysis and pigments quantified using high performance liquid chromatography. Coefficients calculated from the means of three hybrid bermudagrass cultivars across 28 d after withholding irrigation measured in August 2015 at two locations in East Tennessee (n = 18).

	Days After Withholding Water															
	0		4		8		12		16		20		24		28	
Pigments	*r*	*P* > |*r*|	*r*	*P* > |*r*|	*r*	*P* > |*r*|	*r*	*P* > |*r*|	*r*	*P* > |*r*|	*r*	*P* > |*r*|	*r*	*P* > |*r*|	*r*	*P* > |*r*|
Total Chlorophyll	0.46	*	0.60	**	0.56	*	0.68	**	0.91	***	0.81	***	0.80	***	0.89	***
Lutein	0.65	**	0.53	*	0.40	NS	0.56	*	0.79	***	0.72	***	0.73	***	0.84	***
β-carotene	0.67	**	0.56	*	0.71	***	0.68	***	0.90	***	0.81	***	0.58	**	0.66	**
Total Carotenoids	0.62	**	0.53	*	0.53	*	0.61	**	0.86	***	0.78	***	0.68	**	0.87	***

*, **, ***, Significant at $P \leq 0.05$, 0.01, and 0.001, respectively

NS, Not Significant

Table 6. Pearson correlation coefficients (*r*) between digital image analysis color values (Hue, Saturation, and Brightness) and pigments quantified using high performance liquid chromatography (n = 48). Coefficients calculated from the means of three hybrid bermudagrass cultivars measured in August of 2015 at two locations in East Tennessee.

		Pigments									
	DIA	Zeaxanthin		Total Chlorophyll		Lutein		β-carotene		Total Carotenoids	
Cultivar	Color Parameter	*r*	*P* > |*r*|	*r*	*P* > |*r*|	*r*	*P* > |*r*|	*r*	*P* > |*r*|	*r*	*P* > |*r*|
	Hue	-0.62	***	0.86	***	0.64	***	0.85	***	0.78	***
TifTuf	Saturation	-0.68	***	0.84	***	0.62	***	0.85	***	0.76	***
	Brightness	0.45	***	-0.87	***	-0.77	***	-0.90	***	-0.86	***
	Hue	-0.39	**	0.85	***	0.85	***	0.89	***	0.89	***
Latitude 36	Saturation	-0.42	**	0.82	***	0.81	***	0.87	***	0.86	***
	Brightness	0.27	NS	-0.87	***	-0.87	***	-0.89	***	-0.89	***
	Hue	-0.32	*	0.91	***	0.82	***	0.91	***	0.87	***
Tifway	Saturation	-0.43	**	0.86	***	0.74	***	0.86	***	0.81	***
	Brightness	0.16	NS	-0.91	***	-0.88	***	-0.92	***	-0.90	***

*, **, ***, Significant at $P \leq 0.05$, 0.01, and 0.001, respectively

NS, Not Significant

VITA

Cory Alan Yurisic was born on September 24th, 1984 to Karen and Mark Yurisic in Meadville, Pennsylvania. Raised with older siblings, Chad and Carrie, Cory attended Saegertown Jr./Sr. High School in Saegertown, Pennsylvania where he graduated in 2003. Cory attended college as an undergraduate at Gannon University in Erie, Pennsylvania where he studied electrical engineering for two years. After transferring to Middle Tennessee State University in Murfreesboro, Tennessee, Cory continued his undergraduate studies in music recording and construction management. Upon finishing his education at Middle Tennessee State, Cory worked in Dalton, Georgia for a year before deciding to return to school to receive a bachelor's degree in turfgrass science and management at the University of Tennessee in Knoxville, Tennessee. Cory graduated in 2014, and decided to continue his academic career by pursuing a masters degree at the University of Tennessee. He will graduate with his masters degree in the Spring of 2016, after which he has accepted a job at the Scotts Miracle-Gro Company in Apopka, Florida as a research specialist.